Doan *et al. Applied Biological Chemistry* (2024) 67:17
https://doi.org/10.1186/s13765-024-00875-w

KSABC
The Korean Society for Applied Biological Chemistry

ARTICLE

Open Access

Antioxidant activity of different cultivars of *Chrysanthemum morifolium* and quantitative analysis of phenolic compounds by HPLC/UV

Tham Thi Mong Doan[1†], Gia Han Tran[2†], Toan Khac Nguyen[3], Jin Hee Lim[1*] and Sanghyun Lee[2,4*]

Abstract

Chrysanthemum morifolium is classified within the Asteraceae botanical family and serves as a phytomedicine in many countries. The objective of this study was to quantitatively analyze twelve phenolic compounds through HPLC/UV and to assess the antioxidant abilities using the DPPH and $ABTS^+$ assays in the leaves and flowers of six cultivars of *C. morifolium*: 'Geumsu', 'Ilonka', 'Silvia', 'Pompadour', 'Yes Holic', and 'Ford'. The results indicated that the leaves of 'Geumsu' and 'Ford', as well as the 'Pompadour' flowers contained high levels of phenolic compounds and exhibited strong antioxidant abilities. Additionally, a relationship between the phenolic compounds and antioxidant activities was observed. These findings provide foundational knowledge about *C. morifolium* cultivars, which are promising natural sources that can offer health benefits.

Keywords Antioxidant, *C. morifolium*, Cultivar, High-performance liquid chromatography, Phenolic compounds, Quantitative analysis

Introduction

Oxidation constitutes a significant process in food, chemicals, and living systems; however, a by-product is the production of free radicals, particularly reactive oxygen species (ROS) [1]. Furthermore, substantial quantities of ROS are generated within humans through natural physiological processes, external environmental interactions, and dietary habits, which may be harmful [2]. These ROS contributions extend to processes such as food spoilage, degradation of chemical materials, and the onset of over a hundred human disorders [3, 4]. However, the utilization of antioxidant substances can counteract the oxidation process. These compounds, even at low concentrations, substantially delay or entirely prevent the oxidation of easily susceptible substrates [5]. Recently, the isolation, characterization, and widespread application of natural compounds endowed with antioxidant properties have been demonstrated in various medical contexts [6]. Numerous methods are employed to evaluate the efficacy of natural antioxidants, encompassing assays, such as the ferric reducing antioxidant power assay [7], the β-carotene/linoleic acid assay [8], the Rancimat method [9], inhibition of low-density lipoprotein oxidation [10], the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay [11], etc. This assortment of methods is necessitated by the intricate nature of the analyzed substrates, often presenting as complex mixtures comprising numerous

†Tham Thi Mong Doan and Gia Han Tran are equally contributed to this article.

*Correspondence:
Jin Hee Lim
jinheelim@sejong.ac.kr
Sanghyun Lee
slee@cau.ac.kr
[1] Department of Bio-Industry Resources Engineering, Sejong University, Seoul 05006, Republic of Korea
[2] Department of Plant Science and Technology, Chung-Ang University, Anseong 17546, Republic of Korea
[3] Department of Plant Biotechnology, Sejong University, Seoul 05006, Republic of Korea
[4] Natural Product Institute of Science and Technology, Anseong 17546, Republic of Korea

compounds with diverse functional groups, polarities, and chemical behaviors [12].

Phenolic is the most encountered natural antioxidant compound in plants [13]. They possess one or more hydroxyl groups in their structure, allowing for their structural classification into two major classes: phenolic acids and flavonoids [14]. Additionally, phenolic compounds are applied in growth regulation, hormonal activity, pH regulation, antimicrobial effects, metabolism, and the induction of dormant periods [15].

Chrysanthemum morifolium, commonly known as chrysanthemum or florist's chrysanthemum, is a perennial flowering plant belonging to the Asteraceae (Compositae) family [16, 17]. It is a well-known herbal medicine, which is used as a dietary supplement or health tea in numerous Asian countries, including China, Thailand, Japan, and Korea [18, 19]. *C. morifolium* is believed to possess therapeutic properties for treating conditions, such as headache, influenza, hepatic ailments, inflammation, arteriosclerosis, hypertension, hyperuricemia, diabetes alleviation, and eye diseases [20–22]. Furthermore, it has exhibited a range of significant biological characteristics, including antioxidant, antimutagenic, anticancer, anti-inflammatory, antitumor, antibacterial, and antiviral activities [23–25]. These pharmacological attributes are primarily ascribed to its active compounds, which encompass alkanes, phenolic acids, flavonoids, terpenoids, unsaturated fatty acids, and polysaccharides [26, 27]. Presently, diverse cultivars and varieties of *C. morifolium* have been selectively developed for horticultural applications [28]; consequently, the composition of biological compounds and associated activities may undergo modification through plant breeding or hybridization processes.

Therefore, this study was conducted to assess the antioxidant capacity using the DPPH and $ABTS^+$ (2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) assays, as well as to analyze the content of phenolic compounds through high-performance liquid chromatography (HPLC) in different cultivars of *C. morifolium*.

Materials and methods

Plant materials

Six cultivars of *C. morifolium* (Table 1, Fig. 1) were grown by Prof. Jinhee Lim, Sejong University, Republic of Korea, in September 2022. The leaves and flowers of these cultivars were freshly harvested between December 2022 and January 2023 (16-week-old) and dried. Then, they were cut into small pieces prior to extraction. All specimens (S1–S12) were deposited at the herbarium of the Department of Bio-Industry Resources Engineering, Sejong University, Seoul, Republic of Korea.

Table 1 List of six examined *C. morifolium* cultivars

Plant part	Cultivar name	Sample No
Leaf	'Geumsu'	S1
	'Ilonka'	S2
	'Silvia'	S3
	'Pompadour'	S4
	'Yes Holic'	S5
	'Ford'	S6
Flower	'Geumsu'	S7
	'Ilonka'	S8
	'Silvia'	S9
	'Pompadour'	S10
	'Yes Holic'	S11
	'Ford'	S12

Instruments and reagents

HPLC analysis was conducted using an HPLC instrument comprising a Waters Alliance e2695 Separations Module (Waters Corporation, Milford, MA, USA) and a Waters 2489 UV/Vis Detector (Miami, CA, USA). The configuration encompassed a pump and an auto-sampler, integrated with a YMC Pack Pro C18 column (4.6×250 mm, 5 µm). HPLC-grade solvents were procured from J. T. Baker (Philipsburg, Pennsylvania, USA). This selection included water, acetonitrile, and methanol (MeOH). Furthermore, acetic acid was acquired from Samchun Chemicals (Pyeongtaek, Republic of Korea). In the context of the assays, both an Epoch microplate spectrophotometer by BioTek (Winooski, VT, USA), and a microplate reader were utilized. The determination of radical scavenging activity involved the application of DPPH and $ABTS^+$, while potassium persulfate was obtained from Sigma (MA, USA). Additionally, a collection of 12 standard compounds (Fig. 2) was sourced from the Natural Product Institute of Science and Technology (www.nist.re.kr), Anseong, Korea, which encompassed chlorogenic acid (purity: 98.96%) (**1**), schaftoside (purity: 97.02%) (**2**), isoschaftoside (purity: 97.15%) (**3**), luteoloside (purity: 97.73%) (**4**), isochlorogenic acid B (purity: 92.58%) (**5**), isochlorogenic acid A (purity: 98.72%) (**6**), cosmosiin (purity: 99.52%) (**7**), isochlorogenic acid C (purity: 96.93%) (**8**), linarin (purity: 99.28%) (**9**), luteolin (purity: 97.22%) (**10**), apigenin (purity: 98.71%) (**11**), and acacetin (purity: 98.68%) (**12**).

Sample extraction and preparation

A quantity of 5 g of dried samples was subjected to extraction using ethanol (EtOH) in a reflux extractor

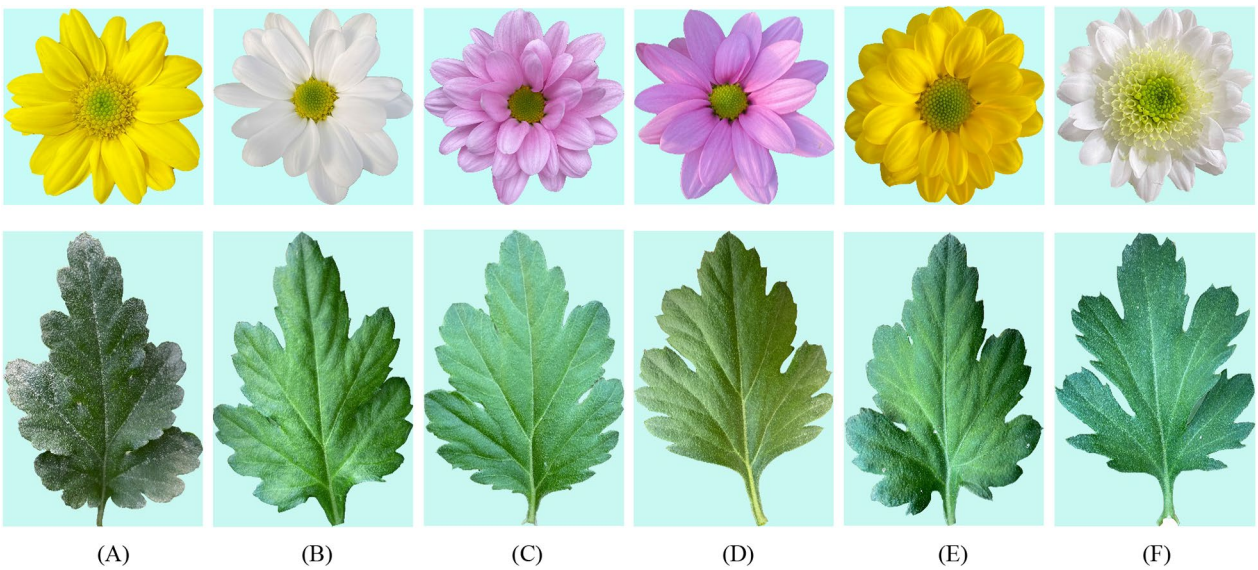


Fig. 1 Plant materials of six cultivars 'Geumsu' (**A**), 'Ilonka' (**B**), 'Silvia' (**C**), 'Pampadour' (**D**), 'Yes Holic' (**E**), and 'Ford' (**F**)

over a period of 3 h. This extraction process was replicated 3 times [29]. Following the utilization of a rotary evaporator, the resulting dehydrated extracts were gathered, and the extraction yield was calculated (Table 2). Each extract (10 mg) was precisely measured and diluted with 1 mL EtOH and distilled water to form a stock for the DPPH and $ABTS^+$ assays, respectively. After filtering through a 0.45 µm membrane filter, sequential dilutions were performed on the stocks to plot a calibration curve for each sample. HPLC analysis preparation involved the extract being dissolved in MeOH, appropriately diluted, and formulated. After dissolution by ultra-sonication, the solution was filtered using a 0.45 µm polyvinylidene fluoride (PVDF) membrane filter to prepare the test solution. A total of 12 standard compounds were precisely weighed at 2 mg and dissolved in 1 mL of MeOH to create stock solutions (2000 ppm) for each standard. After complete dissolution by ultra-sonication, the solutions were filtered using a 0.45 µm PVDF membrane filter.

DPPH radical scavenging activity

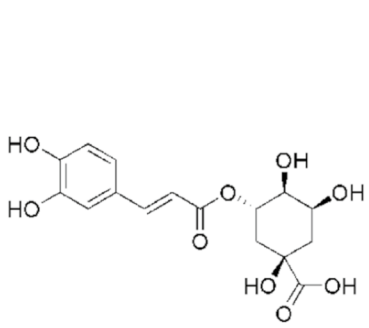
The DPPH radical-scavenging assay commenced by creating a functional solution with a concentration of 0.2 mM DPPH. Here, the original DPPH stock solution was diluted using 95% EtOH. Next, a mixture was prepared by combining 10 µL of the test solution with 200 µL of the DPPH working solution in each well of a 96-well plate. This combination was repeated 3 times to ensure accuracy. Then, the solutions were mixed thoroughly using a microplate shaker and placed in darkness to incubate for a duration of 30 min. Subsequently, the absorbance was measured at a wavelength of 514 nm. The calculation of the DPPH radical-scavenging rate played a pivotal role in constructing the calibration curves. Ascorbic acid was employed as the standard and used as a reference.

$ABTS^+$ radical scavenging activity

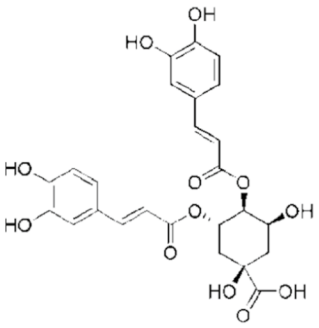
The $ABTS^+$ radical-scavenging assay was performed by diluting the $ABTS^+$ solution with water to create the $ABTS^+$ working solution. Subsequently, each test solution (10 µL) was combined with the $ABTS^+$ working solution (200 µL) and added to each well of a 96-well plate, with the reaction being replicated 3 times for accuracy. The solutions were mixed thoroughly on a microplate shaker and incubated for 30 min in the dark before the absorbance was measured at 734 nm. The $ABTS^+$ radical-scavenging rate calculation was used to construct the calibration curves. Ascorbic acid was employed as the standard for the purpose of comparison.

HPLC condition

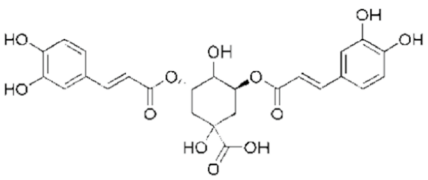
Quantitative analysis of the extracts was conducted using a reverse-phase HPLC system, employing a YMC Pack-Pro C18 column (25 cm × 4.6 mm, 5 µm), and a gradient elution. The mobile phase was composed of 0.25% acetic acid in water (A) and acetonitrile (B), and the elution conditions were 10% B from 0 to 5 min, 20% B at 10 min, 25% B at 20 min, 30% B at 30 min, 40% B at 35 min, and 100% B at 40 min, which was maintained until 45 min. The column temperature was retained at 30 °C, with an injection volume of 10 µL, a flow rate of 1.0 mL/min, and wavelength monitoring set to 356 nm.



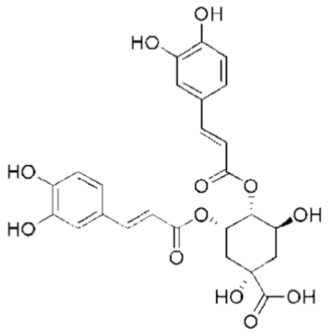
1



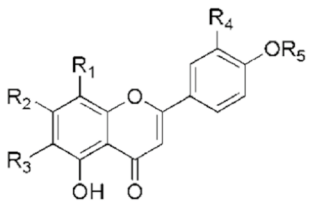
5



6



8



Compound	R_1	R_2	R_3	R_4	R_5
2	Ara	OH	Glc	H	H
3	Glc	OH	Ara	H	H
4	H	O-Glc	H	OH	H
7	H	O-Glc	H	H	H
9	H	O-Rut	H	H	CH_3
10	H	OH	H	OH	H
11	H	OH	H	H	H
12	H	OH	H	H	CH_3

Fig. 2 Chemical structures of 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**)

Calibration curves

The calibration curve was generated by plotting the concentrations of the standard solution against their corresponding peak areas. The evaluation of linearity in this curve relied upon the coefficient of determination (r^2), following which, the calibration curve was used to compute the concentrations of the standards in the samples. The calibration equations were established

Table 2 Extraction yield from 12 samples

Sample No	Dry sample (g)	Extract (g)	Yield (%)
S1	5	1.2	24
S2	5	1.6	32
S3	5	1.5	30
S4	5	1.5	30
S5	5	1.2	24
S6	5	1.3	26
S7	5	1.1	22
S8	5	1.6	32
S9	5	1.6	32
S10	5	1.7	34
S11	5	1.3	26
S12	5	1.2	24

using peak area (Y), concentration (X, mg/mL), and the mean value ± standard deviation (n = 3).

Statistical analysis

All statistical analyses were performed using the software Minitab 16.0. Significant differences between the results were calculated by using anova analysis (ANOVA) and multiple comparisons of the Tukey test, with a significance level of $p < 0.05$.

Results and discussion

DPPH is a chemical compound that is frequently employed in laboratory experiments to evaluate the antioxidant activity of various substances [30]. It is a stable free radical that is characterized by a deep purple color and possesses an unpaired electron, which renders it highly reactive. Antioxidants reduce DPPH by donating an electron, thereby causing a color change to yellow, which can be measured using spectrophotometry [31]. The extent of this color change is directly proportional to the antioxidant activity of the tested compound.

According to the DPPH assay, all the samples exhibited lower antioxidant activities compared to ascorbic acid (Table 3). Among the 12 samples (S1–S12) that were examined, S10 (IC_{50} = 1.2 mg/mL) showed the highest antioxidant capacity, while the lowest was observed in sample S2 (IC_{50} = 7.1 mg/mL). When comparing the radical scavenging activities between the leaf and flower samples from the same cultivar, there were no significant differences in the abilities between the leaves and flowers among four cultivars: 'Geumsu', 'Ilonka', 'Yes Holic', and 'Ford'. However, it is noteworthy that the antioxidant ability of the 'Silvia' and 'Pompadour' flowers was higher than that of their corresponding leaves. Among the leaf samples, S6 (IC_{50} = 2.0 mg/mL) displayed the highest antioxidant capacity, followed by S1 (IC_{50} = 2.5 mg/mL), S5 (IC_{50} = 3.0 mg/mL), S3 (IC_{50} = 3.8 mg/mL), and S4 (IC_{50} = 5.9 mg/mL), with the lowest observed in S2 (IC_{50} = 7.1 mg/mL). Conversely, among the flower samples, S10 (IC_{50} = 1.2 mg/mL) exhibited the highest antioxidant capacity, followed by S12 (IC_{50} = 2.5 mg/mL), S7 (IC_{50} = 2.6 mg/mL), S9 (IC_{50} = 2.6 mg/mL), and S11 (IC_{50} = 3.8 mg/mL), with the lowest observed in S8 (IC_{50} = 7.0 mg/mL). Therefore, overall Ford exhibited the highest antioxidant ability in both its leaves and flowers, whereas Ilonka displayed the weakest ability among the tested cultivars.

$ABTS^+$, like DPPH, is commonly used in laboratory experiments to assess antioxidant activity [32]. When $ABTS^+$ is in its oxidized form, it becomes a stable radical cation with a blue–green color due to unpaired electrons. Antioxidants added to a solution with $ABTS^+$ donate electrons to the radical, reducing it, which causes the color to change from blue–green to colorless [33]. The results of the antioxidant capacity assessment of S1–S12, by the $ABTS^+$ assay (Table 4), closely mirrored those obtained by the DPPH assay. Specifically, the highest antioxidant capacity was observed in S10 (IC_{50} = 1.1 mg/mL), while the lowest was noted in S2 (IC_{50} = 8.9 mg/mL). Similarly, when assessing the antioxidant capacity in the leaf samples, S6 (IC_{50} = 2.1 mg/mL) demonstrated the highest antioxidant capacity, followed by S1 (IC_{50} = 2.7 mg/mL), S5 (IC_{50} = 3.1 mg/mL), S3 (IC_{50} = 4.1 mg/mL), and S4 (IC_{50} = 5.7 mg/mL), with the lowest capacity observed in S2 (IC_{50} = 8.9 mg/mL).

Analysis of the flower samples by both the DPPH and $ABTS^+$ assays revealed that S10 (IC_{50} = 1.1 mg/mL) exhibited the highest antioxidant capacity. Conversely, S11 (IC_{50} = 3.9 mg/mL) and S8 (IC_{50} = 6.1 mg/mL) displayed comparatively lower antioxidant capacities than the other samples. However, there was a slight difference between the two assays. Particularly, in the DPPH assay, which showed that S12 had a higher antioxidant capacity compared to S7 and S9, whereas the $ABTS^+$ assay indicated that S9 (IC_{50} = 2.0 mg/mL) had a higher antioxidant capacity than S12 (IC_{50} = 2.2 mg/mL) and S7 (IC_{50} = 3.3 mg/mL). This disparity can be attributed to various factors, including differences in wavelength, the number of unpaired electrons, and the sensitivity of the assays. Moreover, the DPPH assay contains some drawbacks. One notable characteristic of this assay is its lack of specificity in the assessment of free radical scavenging, as it quantifies scavenged free radicals originating from a spectrum of compounds, thereby encompassing both the phenolic and non-phenolic constituents, such as ascorbic acid [34].

Additionally, comparisons within the same cultivar exhibited that the flower samples of 'Ilonka', 'Silvia', and

Table 3 DPPH radical scavenging activity of S1 – S12

Sample	Concentration (mg/mL)	DPPH	
		Scavenging activity (%)	IC_{50} (mg/mL)
S1	1.25	28.97 ± 2.67	2.5 ± 0.2[de]
	2.5	50.13 ± 5.31	
	5.0	88.73 ± 0.74	
S2	2.5	22.66 ± 1.57	7.1 ± 0.2[a]
	5.0	36.11 ± 2.03	
	10.0	67.46 ± 0.90	
S3	1.25	20.14 ± 2.74	3.8 ± 0.1[c]
	2.5	35.66 ± 1.69	
	5.0	63.02 ± 1.41	
S4	2.5	27.70 ± 0.62	5.9 ± 0.2[b]
	5.0	43.59 ± 2.67	
	10.0	77.68 ± 3.61	
S5	1.25	25.22 ± 3.38	3.0 ± 0.1[d]
	2.5	46.04 ± 2.27	
	5.0	74.45 ± 3.11	
S6	1.25	36.60 ± 1.47	2.0 ± 0.1[e]
	2.5	60.88 ± 2.09	
	5.0	90.58 ± 0.80	
S7	1.25	26.49 ± 1.49	2.6 ± 0.1[d]
	2.5	52.16 ± 0.46	
	5.0	83.47 ± 1.05	
S8	2.5	21.42 ± 2.69	7.0 ± 0.4[a]
	5.0	35.70 ± 3.47	
	10.0	69.30 ± 1.56	
S9	1.25	26.19 ± 3.05	2.6 ± 0.3[d]
	2.5	52.69 ± 6.40	
	5.0	86.43 ± 1.82	
S10	0.5	25.71 ± 2.74	1.2 ± 0.1[f]
	1.0	42.47 ± 1.17	
	2.0	80.23 ± 1.50	
S11	1.25	20.52 ± 2.93	3.8 ± 0.2[c]
	2.5	35.36 ± 2.65	
	5.0	62.83 ± 1.05	
S12	1.25	32.43 ± 0.96	2.5 ± 0.1[de]
	2.5	47.43 ± 3.46	
	5.0	89.18 ± 0.11	
AA			0.14 ± 0.0

AA represents ascorbic acid, which was the positive control

[a–f] Different letters in the same column indicate significant statistical differences ($p < 0.05$)

'Pompadour' provided better antioxidant capacities, whereas 'Geumsu', 'Yes Holic', and 'Ford' displayed higher antioxidant capacities in their leaf samples. Nevertheless, both the DPPH and $ABTS^+$ assays demonstrated that Ford consistently produced the highest antioxidant capacity among the six cultivars in both its flowers and leaves, while 'Ilonka' consistently exhibited the lowest antioxidant capacity.

According to previous research on the antioxidant activity of five fractions (hexane, chloroform, ethyl acetate, butanol, and water) of broccoli MeOH extract, the results indicate that the butanol fraction exhibited

Table 4 $ABTS^+$ radical scavenging activity of S1–S12

Sample	Concentration (mg/mL)	$ABTS^+$	
		Scavenging activity (%)	IC_{50} (mg/mL)
S1	1.25	27.85 ± 0.55	2.7 ± 0.1 [h]
	2.5	49.32 ± 0.44	
	5.0	82.47 ± 0.56	
S2	2.5	20.67 ± 0.27	8.9 ± 0.1[a]
	5.0	34.06 ± 0.91	
	10.0	54.32 ± 0.44	
S3	1.25	23.14 ± 0.42	4.1 ± 0.1[d]
	2.5	36.13 ± 0.60	
	5.0	58.43 ± 0.46	
S4	2.5	27.85 ± 0.15	5.7 ± 0.1[c]
	5.0	47.01 ± 0.80	
	10.0	76.90 ± 0.59	
S5	1.25	25.48 ± 0.80	3.1 ± 0.1 [g]
	2.5	42.91 ± 0.97	
	5.0	73.06 ± 0.36	
S6	1.25	35.59 ± 0.27	2.1 ± 0.1[j]
	2.5	60.23 ± 0.50	
	5.0	93.19 ± 0.44	
S7	1.25	22.37 ± 0.20	3.3 ± 0.1[f]
	2.5	42.44 ± 1.04	
	5.0	70.52 ± 1.10	
S8	2.5	24.11 ± 0.78	6.1 ± 0.1[b]
	5.0	45.21 ± 0.89	
	10.0	74.76 ± 0.50	
S9	1.25	37.03 ± 1.13	2.0 ± 0.1[j]
	2.5	61.47 ± 0.61	
	5.0	92.65 ± 0.15	
S10	0.5	27.55 ± 0.38	1.1 ± 0.1 [k]
	1.0	47.41 ± 1.16	
	2.0	76.96 ± 1.02	
S11	1.25	22.40 ± 0.80	3.9 ± 0.1[e]
	2.5	36.56 ± 0.93	
	5.0	60.77 ± 0.83	
S12	1.25	33.42 ± 0.21	2.2 ± 0.1[i]
	2.5	57.26 ± 0.50	
	5.0	91.05 ± 0.45	
AA [a]			0.11 ± 0.0

[a] AA represents ascorbic acid, which was the positive control

[a–k] Different letters in the same column indicate significant statistical differences ($p < 0.05$)

the highest DPPH (EC_{50} = 0.524 mg/mL) and $ABTS^+$ (EC_{50} = 0.180 mg/mL) radical scavenging activities [35]. Compared to the results of the present study, the antioxidant activities of *C. morifolium* were significantly stronger than broccoli.

Phenolic compounds are renowned for their antioxidative attributes and exhibit a spectrum of biological activities, which can provide potential advantages for human health [36]. They can help protect cells and tissues from oxidative stress by neutralizing harmful free radicals, which can damage DNA, proteins, and lipids. Moreover, the intake of dietary sources abundant in phenolic compounds has been associated with a range of health advantages, including a diminished

susceptibility to chronic ailments, such as cardiovascular diseases, malignancies, and neurodegenerative conditions. These compounds may also possess anti-inflammatory and antimicrobial effects [37, 38].

Quantitative analysis of the content of 12 phenolic compounds was conducted using HPLC/UV. Among these compounds, isoschaftoside was found in trace amounts in four samples (S1, S2, S4, and S6), and was undetectable in the remaining samples. Consequently, only 11 phenolic compounds were quantified. The HPLC chromatogram for all the standard compounds can be seen in Fig. 3. The retention times, calibration equations, and correlation factors for the 11 quantified phenolic compounds are presented in Table 5. In general, these compounds exhibited distinct separation with retention times ranging from 11.35 to 41.26 min. The coefficient of determination (r^2) exceeded 0.9990 for all compounds, thereby demonstrating the excellent linearity of the used quantification method. Additionally, the quantification of the contents of the individual compounds in the samples was calculated by the calibration equation, with Y representing a specific peak area and X denoting the compound concentration (Table 6). The chromatograms for all 12 cultivar samples are provided in Fig. 4.

Overall, only a few of the twelve investigated compounds were detected in all the samples. Among the twelve compounds, only six were present in all samples. These compounds are chlorogenic acid, luteoloside, isochlorogenic acid B, isochlorogenic acid A, isochlorogenic acid C, apigenin, and acacetin. Many previous research studies have reported that the principal phenolic constituents in *C. morifolium* include chlorogenic acid, 3-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, 3,4-di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid, 4,5-di-*O*-caffeoylquinic acid, caffeic acid, luteolin, luteolin-7-*O*-glucopyranoside, apigenin, apigenin-7-*O*-glucoside, acacetin-7-*O*-rutinoside, and acacetin [17–19]. Another study also indicated that *C. morifolium* flowers showed the presence of many flavonoids, such as acacetin, apigenin, chrysin, eriodictyol, luteolin, quercetin, quercitrin, isoquercitrin, hyperoside, catechin, and isocatechin [39].

Table 5 Calibration curve equations for compounds **1**–**12**

Compound	t_R	Calibration equation [a]	Correlation factor, r^2 [b]
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

[a] Y = peak area, X = concentration of the standard (μg/mL)

[b] r^2 = coefficient of determination for five calibration data points (n = 3)

Among the twelve samples analyzed, the highest total phenolic compound content was found in S10 (270.61 mg/g extract), whereas the lowest content was detected in S2 (12.90 mg/g extract). In the leaves and flowers samples, S1 and S10 had the highest total phenolic compound content, while the lowest were in S2 and S8. These results are similar to the results regarding the antioxidant activities mentioned above. It can be seen that the higher the total phenolic compound content, the higher the antioxidant activity. However, there are some differences in the results regarding the content of the plant compounds with antioxidant capacities. Specifically, S1 showed a

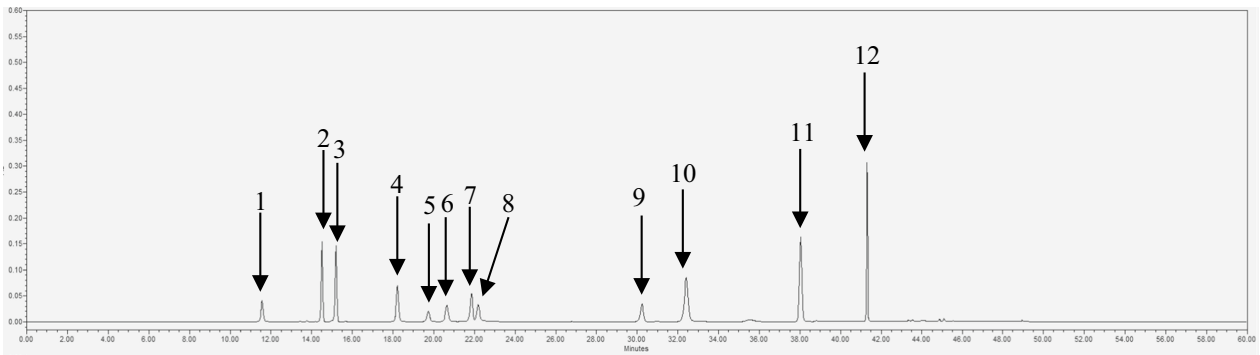


Fig. 3 HPLC chromatogram of standard compounds **1**–**12.** chlorogenic acid (**1**), schaftoside (**2**), isoschaftoside (**3**), luteoloside (**4**), isochlorogenic acid B (**5**), isochlorogenic acid A (**6**), cosmosiin (**7**), isochlorogenic acid C (**8**), linarin (**9**), luteolin (**10**), apigenin (**11**), and acacetin (**12**)

Table 6 Quantification of phenolic compound contents in S1 – S12

Sample	Content (mg/g extract)												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Total**
S1	3.05 ± 0.01^{f}	tr	tr	4.01 ± 0.01^{e}	7.21 ± 0.01^{d}	36.28 ± 0.55^{b}	ND	18.82 ± 0.01^{c}	38.10 ± 0.02^{a}	tr	tr	tr	107.47
S2	0.48 ± 0.01^{g}	0.39 ± 0.01^{h}	tr	1.50 ± 0.01^{c}	0.55 ± 0.01^{f}	6.18 ± 0.01^{a}	ND	1.65 ± 0.01^{b}	tr	0.75 ± 0.01^{e}	0.19 ± 0.01^{i}	1.21 ± 0.01^{d}	12.90
S3	1.86 ± 0.01^{e}	tr	ND	3.56 ± 0.01^{c}	2.46 ± 0.01^{d}	14.34 ± 0.01^{a}	ND	7.10 ± 0.01^{b}	0.72 ± 0.01^{f}	ND	tr	0.34 ± 0.01^{g}	30.38
S4	1.27 ± 0.01^{e}	0.45 ± 0.01^{h}	tr	1.34 ± 0.01^{d}	1.03 ± 0.01^{f}	9.56 ± 0.01^{b}	tr	2.45 ± 0.01^{c}	10.76 ± 0.01^{a}	ND	0.13 ± 0.01^{i}	0.98 ± 0.01^{g}	27.97
S5	1.77 ± 0.01^{d}	ND	ND	3.45 ± 0.01^{c}	1.84 ± 0.01^{d}	15.83 ± 0.02^{a}	0.93 ± 0.04^{f}	4.65 ± 0.11^{b}	ND	1.37 ± 0.01^{e}	0.68 ± 0.01^{g}	0.98 ± 0.01^{f}	31.50
S6	5.82 ± 0.01^{e}	0.82 ± 0.01^{g}	tr	3.80 ± 0.01^{f}	12.23 ± 0.01^{b}	34.07 ± 0.01^{a}	ND	8.49 ± 0.01^{c}	7.51 ± 0.01^{d}	tr	0.03 ± 0.01^{i}	0.24 ± 0.01^{h}	73.01
S7	2.43 ± 0.01^{g}	ND	ND	50.51 ± 0.01^{a}	1.19 ± 0.01^{h}	12.67 ± 0.05^{c}	1.16 ± 0.01^{h}	13.80 ± 0.02^{b}	10.33 ± 0.04^{d}	6.07 ± 0.01^{e}	4.09 ± 0.01^{f}	tr	102.25
S8	2.24 ± 0.01^{c}	ND	ND	9.97 ± 0.03^{a}	tr	7.06 ± 0.01^{b}	ND	1.20 ± 0.01^{e}	tr	1.72 ± 0.01^{d}	0.02 ± 0.01^{f}	tr	22.21
S9	5.14 ± 0.02^{c}	ND	ND	113.78 ± 0.32^{a}	tr	11.20 ± 0.01^{b}	tr	1.63 ± 0.01^{d}	tr	1.30 ± 0.01^{d}	tr	tr	133.05
S10	16.08 ± 0.01^{c}	tr	ND	193.35 ± 0.06^{a}	6.76 ± 0.01^{d}	43.05 ± 0.01^{b}	tr	4.11 ± 0.04^{f}	5.21 ± 0.01^{e}	2.05 ± 0.03^{g}	tr	tr	270.61
S11	2.66 ± 0.01^{d}	tr	ND	34.20 ± 0.01^{a}	tr	7.82 ± 0.01^{b}	1.45 ± 0.01^{e}	0.82 ± 0.01^{f}	ND	3.60 ± 0.01^{c}	0.12 ± 0.01^{g}	tr	50.67
S12	5.23 ± 0.01^{d}	tr	ND	23.01 ± 0.01^{b}	4.50 ± 0.01^{e}	35.83 ± 0.29^{a}	ND	7.33 ± 0.01^{c}	3.24 ± 0.01^{f}	0.80 ± 0.01^{h}	0.28 ± 0.01^{i}	1.08 ± 0.01^{g}	81.30

tr trace, *ND* not detected

[a–i] Different letters in the same row indicate significant statistical differences ($p < 0.05$)

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**)

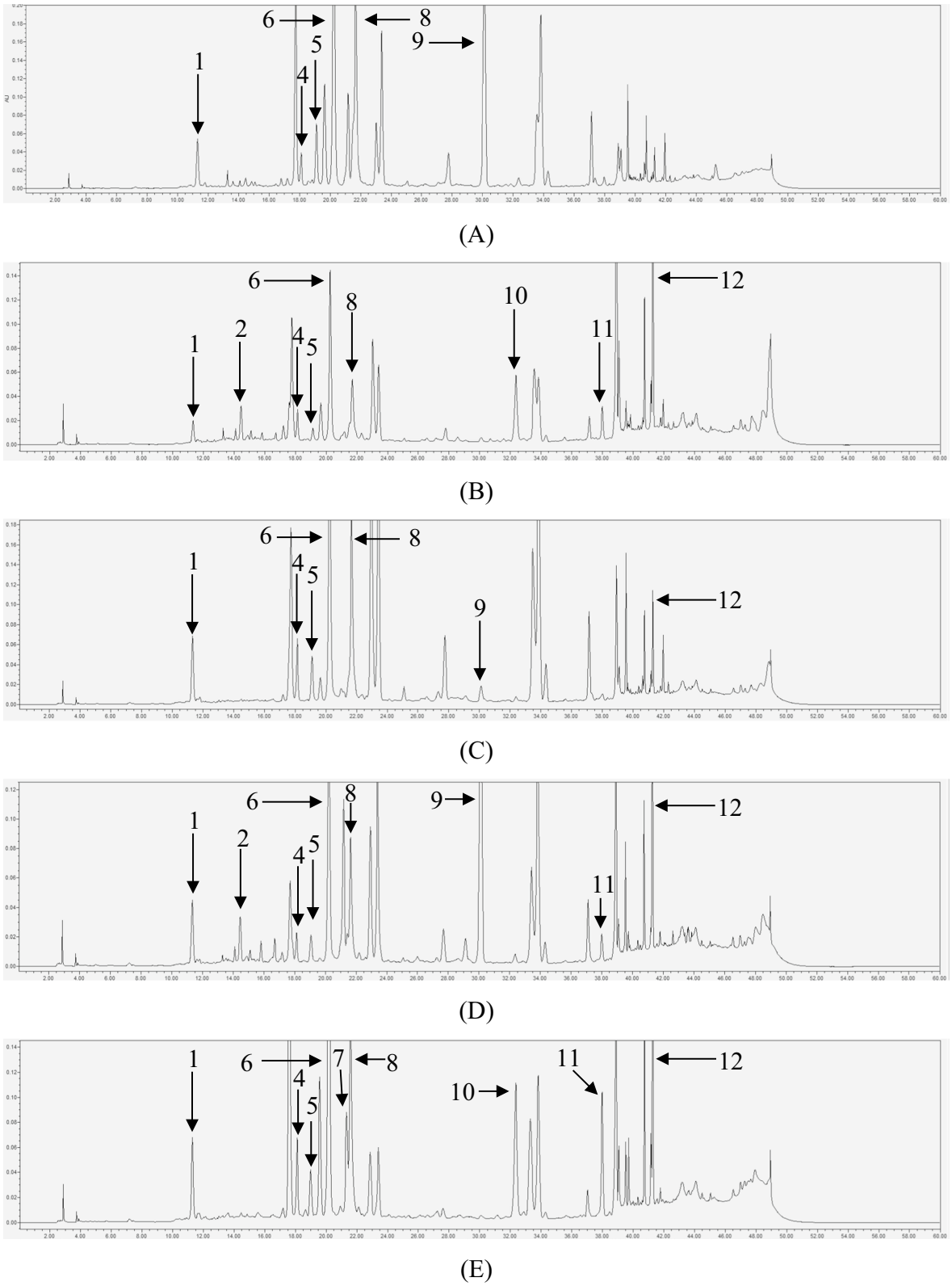
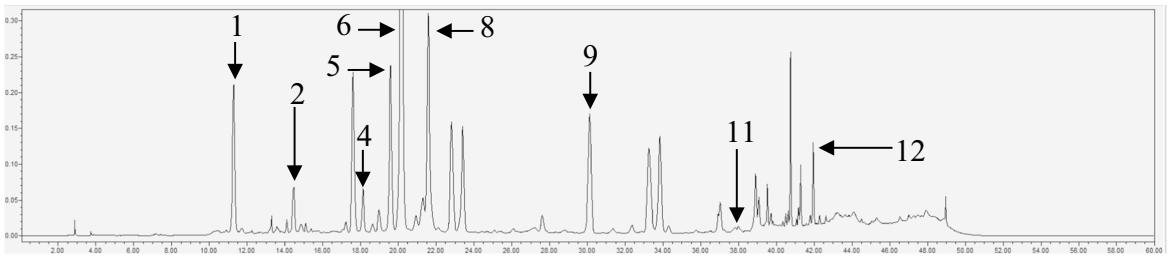
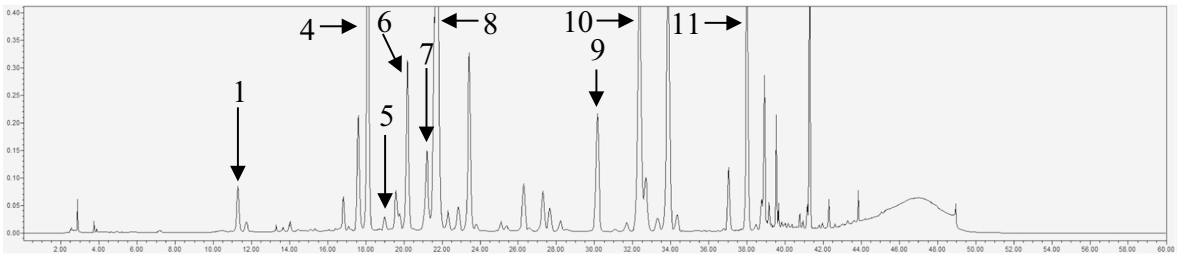


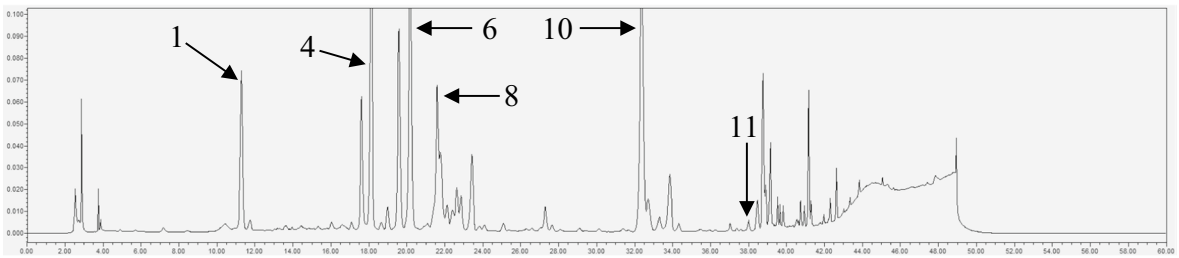
Fig. 4 HPLC chromatograms of S1 (**A**), S2 (**B**), S3 (**C**), S4 (**D**), S5 (**E**), S6 (**F**), S7 (**G**), S8 (**H**), S9 (**I**), S10 (**J**), S11 (**K**), and S12 (**L**). chlorogenic acid (**1**), schaftoside (**2**), isoschaftoside (**3**), luteoloside (**4**), isochlorogenic acid B (**5**), isochlorogenic acid A (**6**), cosmosiin (**7**), isochlorogenic acid C (**8**), linarin (**9**), luteolin (**10**), apigenin (**11**), and acacetin (**12**)



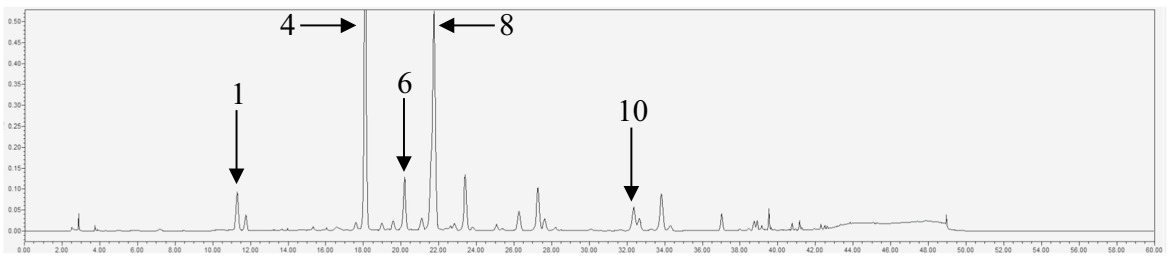
(F)



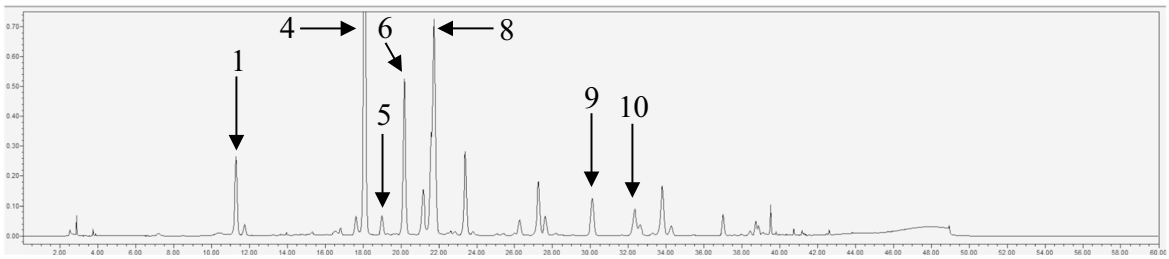
(G)



(H)

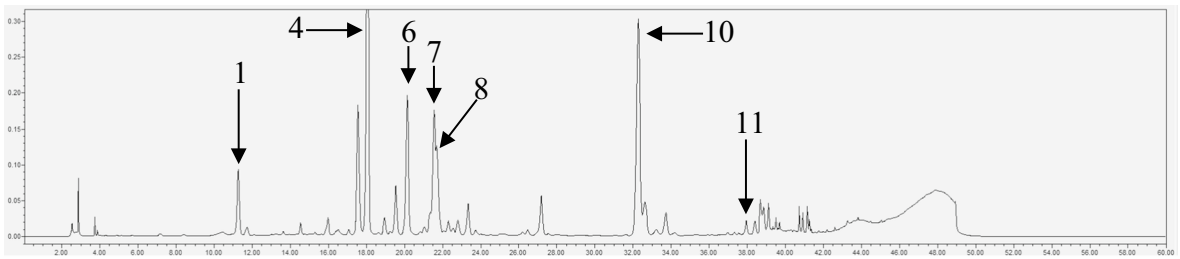


(I)

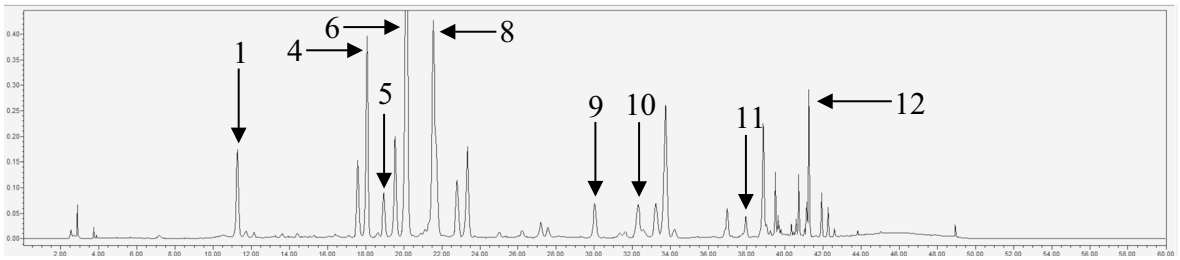


(J)

Fig. 4 continued



(K)



(L)

Fig. 4 continued

higher total phenolic compound content compared to S6, while S9 also had a higher content of phenolic compounds compared to S7, yet S7 had a higher content compared to S12. However, this does not match previous antioxidant activity results, which can be explained as follows: firstly, the antioxidant activity of phenolic compounds can vary significantly depending on the presence and number of hydroxyl groups contained in a compound. Some phenolic compounds may have strong antioxidant properties, while others may have weaker or even pro-oxidant effects [36]. Another reason is that, in this paper, we only examined the content of 12 phenolic compounds. Additionally, there are many other plant compounds that were not investigated in this study, and these compounds could also contribute to the antioxidant capacity.

In a recent investigation, it was documented that dandelion (*Taraxacum mongolicum* Hand.-Mazz.) exhibited a capacity to enhance the exercise performance of mice afflicted with liver dysfunctions [40]. The predominant constituents identified in dandelion included luteolin, rutin, isoquercitrin, myricitrin, chlorogenic acid, gallic acid, caffeic acid, *p*-coumaric acid, protocatechuic acid, and isochlorogenic acid A. These constituents are quite similar to the compounds presented in the six *C. morifolium* cultivars; therefore, it can be expected that in addition to the antioxidant activities, *C. morifolium* may also have medicinal properties similar to dandelions, or other important medicinal properties.

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

Author contributions
TTMD and TKN: Investigation, sample extraction, antioxidant activity, and HPLC analysis, GHT: HPLC analysis, data curation, and writing-original draft, JHL: Conceptualization, funding acquisition, supervision, writing-review and editing, SL: Experimental design, supervision, writing-review and editing.

Declarations

Competing interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Received: 31 December 2023 Accepted: 13 February 2024
Published online: 21 February 2024

References

1. Pourmorad F, Hosseinimehr SJ, Shahabimajd N (2006) Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. African J Biotechnol 5:1142–1145
2. Stobiecka M, Król J, Brodziak A (2022) Antioxidant activity of milk and dairy products. Animals 12:245
3. Huang DJ, Hsien-Jung C, Chun-Der LIN, Yaw-Huei LIN (2005) Antioxidant and antiproliferative activities of water spinach (*Ipomoea aquatica* Forsk) constituents. Bot Bull Acad Sin 46:99–106
4. Sun YM, Wang RX, Yuan SL, Lin XJ, Liu CB (2004) Theoretical study on the antioxidant activity of curcumin. Chinese J Chem 22:827–830
5. Halliwell B (1995) Antioxidant characterization: methodology and mechanism. Biochem Pharmacol 49:1341–1348

6. Mishra K, Ojha H, Chaudhury NK (2012) Estimation of antiradical properties of antioxidants using DPPH assay: a critical review and results. Food Chem 130:1036–1043
7. Benzie IF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 239:70–76
8. Dawidowicz AL, Wianowska D, Baraniak B (2006) The antioxidant properties of alcoholic extracts from *Sambucus nigra* L. (antioxidant properties of extracts). LWT-Food Sci Technol 39:308–315
9. Proestos C, Boziaris IS, Nychas GJ, Komaitis M (2006) Analysis of flavonoids and phenolic acids in Greek aromatic plants: Investigation of their antioxidant capacity and antimicrobial activity. Food Chem 95:664–671
10. Li Y, Guo C, Yang J, Wei J, Xu J, Cheng S (2006) Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. Food Chem 96:254–326
11. Tepe B, Sokmen M, Akpulat HA, Sokmen A (2006) Screening of the antioxidant potentials of six *Salvia* species from Turkey. Food Chem 95:200–204
12. Szabo M, Idițoiu C, Chambre D, Lupea A (2007) Improved DPPH determination for antioxidant activity spectrophotometric assay. Chem Pap 61:214–216
13. Olszowy M (2019) What is responsible for antioxidant properties of polyphenolic compounds from plants? Plant Physiol Biochem 144:135–143
14. Cheynier V, Comte G, Davies KM, Lattanzio V, Martens S (2013) Plant phenolics: recent advances on their biosynthesis, genetics, and ecophysiology. Plant Physiol Biochem 72:1–20
15. Eghbaliferiz S, Iranshahi M (2016) Prooxidant activity of polyphenols, flavonoids, anthocyanins and carotenoids: updated review of mechanism and catalyzing metals. Phytother Res 30:1379–1391
16. Zheng C, Dong Q, Chen H, Cong Q, Ding K (2015) Structural characterization of a polysaccharide from *Chrysanthemum morifolium* flowers and its antioxidant activity. Carbohydr Polym 130:113–121
17. Lin LZ, Harnly JM (2010) Identification of the phenolic components of *Chrysanthemum* flower (*Chrysanthemum morifolium* Ramat). Food Chem 120:319–326
18. Wang S, Hao LJ, Zhu JJ, Zhang QW, Wang ZM, Zhang X, Song XM (2014) Study on the effects of sulfur fumigation on chemical constituents and antioxidant activity of *Chrysanthemum morifolium* cv. Hang-ju. Phytomedicine 21:773–777
19. Ma D, Wako Y (2017) Evaluation of phenolic compounds and neurotrophic/neuroprotective activity of cultivar extracts derived from *Chrysanthemum morifolium* flowers. Food Sci Technol Res 23:457–546
20. Ma YL, Sun P, Feng J, Yue J, Wang Y, Shang YF, Niu XL, Yang SH, Wei ZJ (2021) Solvent effect on phenolics and antioxidant activity of Huangshan Gongju (*Dendranthema morifolium* (Ramat) Tzvel. cv. Gongju) extract. Food Chem Toxicol 147:111875
21. Chen M, Wang K, Zhang Y, Zhang M, Ma Y, Sun H, Sun H (2019) New insights into the biological activities of *Chrysanthemum morifolium*: natural flavonoids alleviate diabetes by targeting α-glucosidase and the PTP-1B signaling pathway. Eur J Med Chem 178:108–115
22. He DX, Ru XC, Wen L, Wen YC, Jiang HD, Bruce IC, Jin J, Ma X, Xia Q (2012) Total flavonoids of Flos Chrysanthemi protect arterial endothelial cells against oxidative stress. J Ethnopharmacol 139:68–73
23. Han Y, Zhou M, Wang L, Ying X, Peng J, Jiang M, Luo G (2015) Comparative evaluation of different cultivars of Flos Chrysanthemi by an anti-inflammatory-based NF-κB reporter gene assay coupled to UPLC-Q/TOF MS with PCA and ANN. J Ethnopharmacol 174:387–395
24. Zhang Y, Li B, Wu G (2022) Dandelion (*Taraxacum mongolicum* Hand.-Mazz.) suppresses the decrease in physical strength and exercise capacity caused by insufficient liver function. Appl Biol Chem 65:89
25. Kim DY, Won KJ, Hwang DI, Lee SY, Choi IH, Kim B, Lee HM (2022) 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. Hortic Environ Biotechnol 63:287–298
26. Tsao R, Attygalle AB, Schroeder FC, Marvin CH, McGarvey BD (2003) Isobutylamides of unsaturated fatty acids from *Chrysanthemum morifolium* associated with host-plant resistance against the western flower thrips. J Nat Prod 66:1229–1231
27. Lu Q, Wang S, Xue S, Yang D, Li L (2020) Effects of drying methods on phenolic components in different parts of *Chrysanthemum morifolium* flower. J Food Proc Preserv 44:e14982
28. Han AR, Kim HY, So Y, Nam B, Lee IS, Nam JW, Jo YD, Kim SH, Kim JB, Kang SY, Jin CH (2017) Quantification of antioxidant phenolic compounds in a new chrysanthemum cultivar by high-performance liquid chromatography with diode array detection and electrospray ionization mass spectrometry. Int J Anal Chem 2017:1254721
29. Tran GH, Uy NP, Kang SH, Heo W, Lee ES, Roh SS, Lee S (2024) HPLC/DAD analysis and antioxidant activity of adlay sprouts and seeds. Separations 11:32
30. Mirzadeh M, Arianejad MR, Khedmat L (2020) Antioxidant, antiradical, and antimicrobial activities of polysaccharides obtained by microwave-assisted extraction method: a review. Carbohydr Polym 229:115421
31. Sharma OP, Bhat TK (2009) DPPH antioxidant assay revisited. Food Chem 113:1202–1205
32. Mareček V, Mikyška A, Hampel D, Čejka P, Neuwirthová J, Malachová A, Cerkal R (2017) ABTS and DPPH methods as a tool for studying antioxidant capacity of spring barley and malt. J Cereal Sci 73:40–45
33. Miller NJ, Rice-Evans CA (1997) Factors influencing the antioxidant activity determined by the $ABTS^{\bullet+}$ radical cation assay. Free Rad Res 26:195–199
34. Suleria HA, Barrow CJ, Dunshea FR (2020) Screening and characterization of phenolic compounds and their antioxidant capacity in different fruit peels. Foods 9:1206
35. Kim JS, Cuong DM, Bae YB, Cho SK (2022) Antioxidant and antiproliferative activities of solvent fractions of broccoli (*Brassica oleracea* L.) sprout. Appl Biol Chem 65:34
36. Gurning K, Lumbangaol S, Situmorang RF, Silaban S (2021) Determination of phenolic contents and antioxidant activity test of ethanol extract of Sirih merah (*Piper crocatum* Ruiz & Pav.) leaves using the DPPH method. J Pendidik Kim 13:137–142
37. Liu J, Yong H, Yao X, Hu H, Yun D, Xiao L (2019) Recent advances in phenolic–protein conjugates: synthesis, characterization, biological activities, and potential applications. RSC Adv 9:35825–35840
38. Kim J, Kim JH, Bang SI, Shin H, Cho EJ, Lee S (2022) Antioxidant activity of edible sprouts and phytosterol contents by HPLC/UV analysis. Hortic Environ Biotechnol 63:769–778
39. Kandylis P (2022) Phytochemicals and antioxidant properties of edible flowers. Appl Sci 12:9937
40. Zhang Z, Zhang Y, Wang L, Cui T, Wang Y, Chen J, Li W (2022) Online screening of natural antioxidants and the antioxidant activity prediction for the extracts from flowers of *Chrysanthemum morifolium* Ramat. J Ethnopharmacol 294:115336

Publisher's Note