Choi *et al. Applied Biological Chemistry* (2023) 66:61
https://doi.org/10.1186/s13765-023-00820-3

KSABC
The Korean Society for Applied Biological Chemistry

ARTICLE

Open Access

Antioxidative phenolic compounds from the aerial parts of *Cyperus exaltatus* var. *iwasakii* and their HPLC analysis

Jungwon Choi[1†], Hak-Dong Lee[1,2†], Hyejin Cho[1], Chang-Dae Lee[1], Gia Han Tran[1], Hoon Kim[3], Sung-Kwon Moon[3] and Sanghyun Lee[1,2,4*]

Abstract

The constituents and antioxidant activities of *Cyperus exaltatus* var. *iwasakii* (CE) have not been studied to date. In this study, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6 sulfonic acid) (ABTS) assays were used to evaluate the radical-scavenging activities of the ethanol extract, four fractions, and isolated compounds of CE. In addition, phenolic acids and flavonoids were isolated from the ethanol extract of CE using column chromatography. The compounds identified by spectroscopy were gallic acid, protocatechuic acid, vanillic acid, *p*-coumaric acid, rutin, ferulic acid, isoquercitrin, astragalin, quercetin, luteolin, apigenin, tricin, and kaempferol. Quantitative analysis using high-performance liquid chromatography (HPLC) revealed that the major flavonoids of CE were astragalin and tricin and that the major phenolic acid was *p*-coumaric acid. In addition, comparative analysis of CE from Ganghwa and Hampyeong habitats using HPLC showed that the Hampyeong CE had a higher phytochemical content. Comparative analyses of the isolated compounds were also conducted among five *Cyperus* species. The highest antioxidant activities were found in the ethyl acetate (EtOAc) fraction, and among the compounds isolated from CE, vanillic acid and quercetin showed remarkable antioxidant activity even when compared with ascorbic acid. The results demonstrate the usefulness of CE, which has not been sufficiently studied previously, and will facilitate the evaluation of its potential effectiveness as antioxidant functional plant material.

Keywords *Cyperus exaltatus*, NMR, HPLC, Antioxidant, Chemical profiling

Introduction

Natural products are an essential source of potential drug intermediates for developing new drugs or health functional supplements. One of the many approaches employed in research of natural products is to select plants that have been commonly used since ancient times but have not been pharmacologically evaluated for possible antioxidant properties. These investigations should aim to identify the various biological functions and components of plant materials. Almost all *Cyperus* species are classified as weeds in agriculture, and herbicide development to remove them has been studied. Since *Cyperus* species are considered weeds, little research has been conducted on them, particularly in terms of their phytochemical composition and bioactivity [1–3].

†Jungwon Choi and Hak-Dong Lee have contributed this article.

*Correspondence:
Sanghyun Lee
slee@cau.ac.kr
[1] Department of Plant Science and Technology, Chung-Ang University, Anseong 17546, Republic of Korea
[2] Natural Product Institute of Science and Technology, Anseong 17546, Republic of Korea
[3] Department of Food and Nutrition, Chung-Ang University, Anseong 17546, Republic of Korea
[4] BET Research Institute, Chung-Ang University, Anseong 17546, Republic of Korea

Among *Cyperus* species, *Cyperus exaltatus* var. *iwasakii* (CE) is a sedge of the family of Cyperaceae and is commonly found in East Asia, Australia, Africa, and North America. CE culms have a triangular cross-section, a smooth surface, and a growing height of 100–180 cm. The leaves are 5–15 mm long, the spikelets are flat, and their color is yellow to dark yellowish-brown [4]. In Africa, the rhizome of CE has been powdered and used to treat pus or anemia caused by malaria [5]. In many countries, including the Republic of Korea, tough culms of CE have been used to make a variety of household products as well as cushions, slats, and huts. Thus, CE is a representative industrial crop among sedges that is used to make daily necessities. In Korea, the main CE production regions are Hampyeong and Ganghwa, and since much of the focus on CE since ancient times has been on its usefulness for making local products, its pharmacological aspects have rarely been explored.

Reactive oxygen species (ROS), such as singlet oxygen ($^1O_2$), superoxide anion (O_2^-), and hydroxyl ($^{\bullet}OH$) radical, and non-free radical species, such as hydrogen peroxide (H_2O_2), are frequently produced as byproducts of biological reactions or external stimuli [6, 7]. ROS have been shown to exert both positive and harmful effects. In some signal transduction pathways, ROS may function as second messengers at extremely low concentrations [8]. However, excess generation of ROS is regarded as the primary cause of oxidative stress as a result of imbalanced antioxidant defense systems, and the creation of ROS may damage important biomolecules such as DNA, lipids, and proteins [9, 10]. Finally, oxidative stress causes the onset of age-related diseases such as cancer, hypertension, atherogenesis, Parkinson's disease, and Alzheimer's disease [11]. Recent studies have shown that antioxidants derived from plants with free radical-scavenging characteristics may play a key role as therapeutic agents in the aging process and free radical-mediated illnesses such as neurodegeneration [12].

Plants have an important function in sustaining human health and improving human quality of life. Plant extracts and phytochemicals such as flavonoids and other polyphenolic compounds have been demonstrated to exhibit biological activity in vitro and in vivo, justifying traditional medicine research focused on the characterization of the biological activity of these plants [13]. Due to their diverse pharmacological properties, including antimicrobial, antioxidant, anticancer, analgesic, anti-inflammatory, and apoptosis-inducing properties, plant-derived natural products, such as polyphenols, tannins, terpenes, alkaloids, and flavonoids, have garnered considerable attention in recent years [14–16]. Many synthetic antioxidant molecules have been demonstrated to be harmful and/or to have mutagenic properties, which has piqued the curiosity of numerous researchers in natural antioxidants [17, 18]. Thus, research into natural antioxidants has become a crucial topic. In light of its widespread application and chemical composition, the in vitro antioxidative activity of the extract, fractions, and isolated compounds of CE is worth evaluation.

In this study, various experiments were performed with CE, which is mainly used as a fiber and industrial crop, to broaden knowledge of its antioxidant properties and phytochemical components.

Materials and methods

Plant materials

The aerial parts of CE were collected from Hampyeong, Korea in 2019. The methanol (MeOH) extracts of CE, *C. difformis* (CD), *C. microiria* (CM), *C. sanguinolentus* (CS), and *C. cyperoides* (CC) were obtained from KRIBB in Daejeon, Korea. We also acquired CE grown in Ganghwa in 2019 for comparative analysis.

Apparatus and chemicals

Open-column chromatography was performed on silica gel (60–200 Mesh ASTM, Germany) with Sephadex LH-20 (Sigma-Aldrich, St. Louis, MO, USA) and LiChroprep RP-18 (Sigma-Aldrich, St. Louis, MO, USA). Chromatographic analysis was performed using a high-performance liquid chromatography (HPLC) system (Agilent Technology 1260 Infinity II; Santa Clara, CA, USA) and INNO C18 column (250×4.6 mm, 5 μm) equipped with a pump, an auto-sampler, and a diode array detector (DAD). The solvents ethanol (EtOH), methanol (MeOH), *n*-hexane, chloroform ($CHCl_3$), acetic acid, ethyl acetate (EtOAc), and *n*-butanol (*n*-BuOH) used for extraction and fractionation were purchased from Samchun Pure Chemicals Co. (Pyeongtaek, Korea). Extraction and evaporation were performed in an EYELA apparatus (Japan) and with the EYELA rotary evaporator system (Japan). In addition, 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was purchased from Roche Diagnostics GmbH (Mannheim, Germany), and 2,2-diphenyl-1-picrylhydrazyl (DPPH, 95%) was purchased from Alfa Aesar (Haverhill, USA).

DPPH radical-scavenging activity

The DPPH method is widely used to measure the antioxidant activity of water-soluble or organic solvent extracts from natural products. In this experiment, the antioxidant activity of extracts and fractions of CE was measured using the method described by Choi et al. [19]. First, 200 μL of 2 mM DPPH dissolved in 95% ethanol and 10 μL of each experimental group were added to an E.P. tube, vortexed, and reacted in the dark for 30 min. The concentrations of the remaining radicals were measured

using a microplate reader (514 nm). Ascorbic acid (Sigma, USA) was used as a positive control. The scavenging ability (IC_{50}) of the extract against DPPH was expressed as the concentration required to reduce the absorbance of the control group using only the solvent by 50%.

$$\text{DPPH radical - scavenging activity (\%)} = (\text{Blank O.D} - \text{Sample O.D}) / \text{Blank O.D} \times 100 \quad (1)$$

ABTS radical-scavenging activity

The ABTS radical-scavenging activity of extracts and fractions of CE was measured by modifying a previously reported method [20]. For the experimental method, 7.4 mM ABTS and potassium persulfate (2.6 mM) dissolved in distilled water were added in a 1:1 ratio and diluted with distilled water (pH 7.4) to an absorbance value of 1.00 ± 0.02. After leaving the mixture in the dark for 24 h, 10 µL of each sample prepared by concentration in 200 µL of the radical stock solution was added. After leaving the mixture for 30 min, the concentrations of the remaining radicals were measured using a microplate reader (734 nm). Ascorbic acid was used as a positive control. The concentration range was measured to be 0.5–0.025 mg/mL, and the scavenging ability (IC_{50}) of the extract against ABTS was expressed as the concentration required to reduce the absorbance of the control group by 50% using only the solvent.

$$\text{ABTS radical - scavenging (\%)} = (\text{Blank O.D} - \text{Sample O.D}) / \text{Blank O.D} \times 100 \quad (2)$$

Total polyphenol content analysis

Total polyphenol content analysis was performed by modifying a previously reported Folin-Ciocalteu method [21] First, 60 µL of 2N Folin-Ciocalteu phenol reagent (St. Lewis, Sigma-Aldrich, USA) was added to the extract. Then, after adding 60 µL of 15% Na_2CO_3 to the solution for 30 min, absorbance was measured at 760 nm using a microplate reader (Epoch, BioTek, Winooski, Vietnam, USA). Finally, a calibration curve was created using gallic acid as the standard to quantify the total polyphenol content.

Extraction, fractionation, and isolation of CE

The dried aerial parts of CE (4.5 kg) from Hampyeong were cut into small pieces and extracted under reflux with 95% ethanol at 80–84℃ for three hours; this process was repeated three times with an equipped extractor. Using a rotary evaporator, the resultant extract solution was filtered and concentrated to obtain a crude ethanol extract (930 g). The ethanol extract of CE (920 g) was suspended in distilled water and subsequently partitioned with *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH to obtain the *n*-hexane (355 g), $CHCl_3$ (25 g), EtOAc (14 g), and *n*-BuOH fractions (40 g). The EtOAc fraction (13.1 g) of CE was placed onto a silica gel column (6 × 80 cm) with a step gradient of $CHCl_3$:MeOH (10:0 to 4:6) and pooled to produce a fraction. The fractions were analyzed and combined using thin layer chromatography to create six additional subfractions. These additional fractions were purified using Sephadex LH-20 column and then dissolved at a step gradient of H_2O:MeOH (9:1–0:1 v/v) to produce compounds **1**, **7**, **8**, **9**, **10**, **11**, **12**, and **13**. The *n*-BuOH fraction (36 g) of CE was also eluted on an open column (8 × 100 cm) using a previously described method. The *n*-BuOH fraction revealed four fractions and recrystallized compounds **2**–**6**.

Compound **1**: White powder; EI-MS: *m/z* 170 $[M]^+$ (100), 162 (40.0), 133 (50.0), 90 (24.0), 80 (12.0); ^{1}H−NMR (500 MHz, DMSO-d_6): δ 7.10 (2H, s, H−1, H−5); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 110.8 (C-2, C-6), 122.4 (C-1), 139.3 (C-4), 146.4 (C-3, C-5), 168.7 (C-7) (Fig. S1 and S2).

Compound **2**: White crystal; EI-MS: *m/z* 154 $[M]^+$ (100), 162 (40.0), 135 (50.0), 90 (25.0), 76 (12.0); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 7.43 (d, 1H, J = 2.0 Hz, H-2), 7.38 (dd, 1H, J = 8.0, 2.0 Hz, H-6), 6.77 (d, 1H, J = 8.0 Hz, H-5); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 172.0 (C = O), 150.7 (C-4), 146.2 (C-3), 125.5 (C-6), 123.7 (C-1), 117.8 (C-2), 115.6 (C-5) (Fig. S3 and S4).

Compound **3**: White powder; EI-MS: *m/z* 168 $[M]^+$ (100), 284 (65.0), 258 (56.0), 168 (13.0), 137 (15.0), 119 (15.0), 83 (70.0), 65 (75.0); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 7.54 (1H, d, J = 2.0 Hz, H-2), 7.55 (1H, dd, J = 8.0, 2.0 Hz, H-6), 6.82 (1H, d, J = 8.0 Hz, H-5), 4.00 (3H, s, OCH_3); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 166.5 (COOH), 152.3 (C-3), 148.4 (C-4), 123.0 (C-1), 122.0 (C-6), 113.4 (C-2), 111.9 (C-5), 54.1 (OCH_3) (Fig. S5 and S6).

Compound **4**: White powder; EI-MS: *m/z* 164 $[M]^+$ (100), 257 (12.0), 228 (5.0), 181 (2.0), 154 (20.0), 128 (11.0), 106 (1.0), 88 (1.0), 70 (4.0); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 7.51 (3H, t, J = 8.0, 15.0 Hz, H-3, H-5, H-9), 6.75 (2H, d, J = 8.0 Hz, H-6, H-8), 6.21 (1H, d, J = 16.5 Hz, H-2); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 168.1 (C-9), 158.3 (C-4), 143.2 (C-7), 131.4 (C-2, C-6), 124.2 (C-1), 113.4 (C-3, C-5), 114.3 (C-8) (Fig. S7 and S8).

Compound **5**: Yellow amorphous powder; FAB-MS: *m/z* 625. ^{1}H-NMR (DMSO-d_6, 500 MHz): δ = 6.18(1H, d, J = 2.0 Hz, H-6), 6.34(1H, d, J = 2.0, H-8), 7.49(1H, d, J = 2.0, H-2′), 6.79(1H, d J = 9.0, H-5′), 7.53(1H, dd, J = 9.0, 2.0, H-6′), 12.53(1H, s, 5-OH), 5.30(1H, d, J = 7.0, H-1′′), 4.34(1H, d, J = 2.0, H-1′′′), 1.00(3H, d, J = 6.0,

H-6‴). ^{13}C-NMR (125 MHz, DMSO-d_6): δ 156.4 (C-2), 131.2 (C-3), 176.3 (C-4), 156.5 (C-5), 100.6 (C-6), 164.4 (C-7), 93.6 (C-8), 161.2 (C-9), 104.1 (C-10), 121.6 (C-1′), 115.1 (C-2′), 144.7 (C-3′), 148.5 (C-4′), 116.1 (C-5′), 120.9 (C-6′), 101.2 (C-1″), 71.8 (C-2″), 74.1 (C-3″), 71.6 (C-4″), 72.8 (C-5″), 67.0 (C-6″) 103.8 (C-1‴), 70.0 (C-2‴), 70.2 (C-3‴), 70.5 (C-4‴), 68.2 (C-5‴), 17.7 (C-6‴) (Fig. S9 and S10).

Compound **6**: Yellow amorphous powder; EI-MS: *m/z* 194 $[M]^+$ (100), 180 (25), 160 (7), 134 (32), 101 (14), 90 (15), 71(27), 52(15); ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 3.94 (3H, s, H-4'), 6.32 (1H, d, J = 14.0 Hz, H-2′), 6.85 (1H, d, J = 9.0 Hz, H-6), 7.14 (1H, dd, J = 8.0, 2.0 Hz, H-5), 7.01 (1H, d, J = 2.0 Hz, H-3), 7.74 (1H, d, J = 14.0 Hz, H-1′); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 55.0 (C-4′), 108.3 (C-5), 114.5 (C-2), 114.8 (C-2'), 124.6 (C-3), 124.7 (C-4), 142.8 (C-1′), 147.0 (C-6), 148.3 (C-1), 171.3 (C-3′) (Fig. S11 and S12).

Compound **7**: Yellow powder; FAB-MS: *m/z* 464. ^{1}H-NMR (DMSO-d_6, 500 MHz): δ 12.65 (5-OH), 7.58 (1H, d, J = 1.5 Hz, H-2′), 7.57 (1H, d, J = 2.0 Hz, H-6′), 6.85 (1H, dd, J = 1.5, 7.5 Hz, H-5′), 6.41 (1H, d, J = 2.0 Hz, H-8), 6.21 (1H, d, J = 2.0 Hz, H-6), 5.47 (1H, d, J = 7.0 Hz, H-1″), 3.60–3.10 (6H, m, H-2″-6″). ^{13}C-NMR (125 MHz, DMSO-d_6): δ 155.1 (C-2), 133.4 (C-3), 177.3 (C-4), 162.3 (C-5), 98.6 (C-6), 165.1 (C-7), 93.4 (C-8), 156.2 (C-9), 104.9 (C-10), 121.6 (C-1′), 115.1 (C-2'), 143.7 (C-3'), 148.5 (C-4′), 116.1 (C-5′), 120.9 (C-6′), 100.7 (C-1″), 74.2 (C-2′), 76.4 (C-3′), 70.0 (C-4′), 77.7 (C-5′), 61.0 (C-6′) (Fig. S13 and S14).

Compound **8**: Yellow powder; ESI−MS: *m/z* 447.2 $[MH]^-$. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 8.06 (2H, dd, J = 12.0, 2.5 Hz, H-2′, H-6'), 6.87 (2H, dd, J = 9.5, 2.5 Hz, H-3', H-5'), 6.40 (1H, d, J = 2.0 Hz, H-8), 6.18 (1H, d, J = 2.0 Hz, H-6), 5.15 (1H, d, J = 7.8 Hz, H-1″), 3.76 (1H, dd, J = 14.0, 4.2 Hz, H-6″b), 3.57 (1H, dd, J = 12.0, 4.5 Hz, H-6″a), 3.49 (1H, d, J = 11.4 Hz, H-3″), 3.40 (1H, d, J = 11.4 Hz, H-2″), 3.27 (1H, d, J = 7.8 Hz H-5″). ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 178.3 (C-4), 163.6 (C-7), 161.8 (C-2), 160.2 (C-5), 157.6 (C-9), 157.3 (C-4'), 134.1 (C-3), 130.9 (C-2', C-6'), 121.2 (C-1'), 114.7 (C-3', C-5'), 104.8 (C-10), 103.5 (C-1″), 98.5 (C-6), 93.3 (C-8), 75.7 (C-5″). 73.8 (C-3″), 71.6 (C-2″), 68.6 (C-4″), 60.5 (C-6″) (Fig. S15 and S16).

Compound **9**: Yellow powder; ESI−MS: *m/z* 301.1 $[MH]^-$ ($C_{15}H_{10}O_7$). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 7.69 (1H, d, J = 2.1 Hz, H-2'), 7.60 (1H, dd, J = 8.4, 2.1 Hz, H-6′), 6.88(1H, d, J = 8.4 Hz, H-5'), 6.46 (1H, d, J = 2.0 Hz, H-8), 6.14 (1H, d, J = 2.0 Hz, H-6). ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 175.9 (C-4), 163.3 (C-7), 161.3 (C-9), 157.8 (C-5), 148.3 (C-4'), 146.5 (C-1), 143.0 (C-1'), 135.8 (C-3) 122.7 (C-1'), 120.2 (C-6'), 115.0 (C-5'), 113.5 (C-2'), 103.3 (C-10), 98.0 (C-6), 93.1 (C-8) (Fig. S17 and S18).

Compound **10**: Yellow powder; ESI−MS: *m/z* 270 $[MH]^-$. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 7.41(1H, dd, J = 8.2, 2.2 Hz, H-6'), 7.50(1H, d, J = 2.2 Hz, H-2′), 6.90(1H, d, J = 8.3 Hz, H-5'), 6.68(1H, s, H-3), 6.45(1H, d, J = 2.0 Hz, H-8), 6.21(1H, d, Hz, H-6); ^{13}C-NMR(125 MHz, DMSO-d_6) δ: 181.7(C-4), 164.1(C-2), 163.9(C-7), 162.4(C-5), 156.2(C-9), 149.7(C-4′), 145.7(C-3′), 121.6(C-1′), 118.9(C-6′), 116.0(C-5′), 113.3(C-2′), 103.7 (C-10), 98.8(C-6), 93.8(C-8) (Fig. S19 and S20).

Compound **11**: Yellow powder; ESI−MS: *m/z* 270 [MH]. ^{1}H-NMR (DMSO-d_6, 500 MHz): 7.83 (2H, d, J = 8.8 Hz, H-2', H-6'), 6.93 (2H, d, J = 8.8 Hz, H-3′, H-5′), 6.83 (1H, d, J = 2.0 Hz, H-6), 6.71 (1H, d, J = 2.0 Hz, H-8), 6.57 (1H, s, H-3); ^{13}C-NMR(125 MHz, DMSO-d_6): 180.4 (C-4), 165.9 (C-5), 164.4 (C-2), 162.5 (C-4′), 160.7 (C-9), 160.1 (C-7), 129.3 (C-2′, C-6′), 123.2 (C-10), 117.1 (C-3′, C-5′), 109.3 (C-10), 106.6 (C-3), 104.7 (C-6), 99.3 (C-8) (Fig. S21 and S22).

Compound **12**: Yellowish white powder; EI-MS *m/z* 330.0740 $[M]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 12.97 (1H, 5-OH), H-7.32 (2H, s, H-2′, H-6′) 6.98 (1H, s, H-3), 6.56 (1H, d, J = 3.0 Hz, H-8), 6.21 (1H d, J = 3.0 Hz, H-6), 3.89 (6H, s, 3′, 5′-OCH_3); ^{13}C-NMR (125 MHz, DMSO-d_6) δ 182.1 (C-4), 164.6 (C-7), 164.1 (C-2), 161.8 (C-5), 157.7 (C-8a), 148.6 (C-3′, C-5′), 140.2 (C-4′), 120.8 (C-1′), 104.7 (C-2′, C-6′), 104.0 (C-4a, C-3), 99.3 (C-6), 94.7 (C-8), 56.8 (C-OCH_3 × 2) (Fig. S23 and S24).

Compound **13**: Yellow powder; EI-MS *m/z* $[M+H]^+$ m/z 287.0546. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 12.48 (1H, s, 5-OH), 10.84 (1H, s, 7-OH), 10.13 (1H, s, 4′-OH), 8.04 (2H, d, J = 9.0 Hz, H-2′, H-6′), 6.95 (2H, d, J = 9.0 Hz, H′3′, H-5′), 6.45 (1H, d, J = 2.0 Hz, H-8), 6.22 (1H, d, J = 2.2 Hz, H-6). ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 176.4 (C-4), 164.4 (C-7), 161.1 (C-5), 159.7 (C-4′), 156.6 (C-9), 147.2 (C-2), 136.1 (C-3), 130.0 (C-2′, C-6′), 122.2 (C-10), 116.0 (C-3′, C5), 103.5 (C-1′), 96.7 (C-6), 94.0 (C-8) (Fig. S25 and S26).

Preparation of samples for HPLC and calibration curves

Two types of CE are cultivated in Hampyeong and Ganghwa, and both were used to prepare MeOH extracts (50 mg/mL). The same sample-preparation method was used to prepare MeOH extracts of four *Cyperus* species. Standard flavonoid and phenolic acid solutions were also prepared by dissolving the respective compounds in MeOH (0.5 mg/mL). Before analysis, all samples were filtered through a syringe filter (0.45 μm, PVDF) using sonication for 20 min. The compounds were diluted in series to produce different concentrations (0.03125–0.5 mg/mL). The calibration curve for each standard was constructed by plotting the concentration (X, μg/mL) versus the peak area (Y). The correlation coefficient (r^2) was used to determine linearity.

HPLC–DAD chromatographic condition

Chromatographic separation of individual compounds was performed using the INNO C18 column (250 × 4.6 mm, 5 μm). The mobile phase was 0.5% trifluoroacetic acid in 10% ACN (0.5:90:10, TFA/ACN/water) (sol A) and 0.5% TFA in 90% ACN (0.5:90:10, TFA/ACN/water) (sol B). The gradient elution system was as follows: 0 min, 90% A; 15 min, 90% A; 18 min, 83% A; 30 min, 80% A; 35 min, 70% A; 55 min, 20% A; 58 min, 100% B; 63 min, 100% B; 65 min, 90% A; and 70 min, 90% A. The injection volume was 10 μL, and the flow rate was 0.8 mL/min. The UV detection wavelength was 270 nm. All injections were performed three times.

Results and discussion

Identification of phytochemical constituents from CE

The results for total polyphenol content and the ABTS and DPPH radical-scavenging activities are shown in Table 1. The EtOAc and *n*-BuOH fractions showed the highest radical-scavenging activity.

For fractions with a high polyphenol content and antioxidant activity, phytochemical isolation was conducted using open-column chromatography. Recrystallized phytochemicals from the EtOAc and *n*-BuOH fractions of CE were identified by nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS). Spectroscopic data from the isolated compounds showed characteristic signals of flavonoids and phenolic acids. Thus, 13 compounds (Fig. 1), namely, gallic acid (**1**) [22], protocatechuic acid (**2**) [23], vanillic acid (**3**) [24], *p*-coumaric acid (**4**) [25], rutin (**5**) [26], ferulic acid (**6**) [27], isoquercitrin (**7**) [28], astragalin (**8**) [29], quercetin (**9**) [30], luteolin (**10**) [31], apigenin (**11**) [32], tricin (**12**) [33], and kaempferol (**13**) [34] were identified using previously published literature. Although none of the 13 compounds isolated from CE are new, this is the first report to describe their isolation from CE. Therefore, our results can be used as a reference for further research on CE.

Table 1 DPPH and ABTS radical-scavenging IC_{50} values and total polyphenol content of extracts and fractions of CE collected from Hampyeong

Sample	IC_{50} (mg/mL)		Total polyphenol (mg GAE/g ext.)
	DPPH assay	ABTS assay	
EtOH extract	3.808 ± 0.114	2.279 ± 0.156	11.92 ± 0.79
n-hexane fr	5.429 ± 0.187	9.969 ± 0.218	12.23 ± 0.00
$CHCl_3$ fr	3.608 ± 0.099	2.987 ± 0.166	7.76 ± 0.46
EtOAc fr	0.793 ± 0.036	0.312 ± 0.007	36.77 ± 0.41
n-BuOH fr	3.348 ± 0.852	1.500 ± 0.097	30.10 ± 0.50
Ascorbic acid	0.187 ± 0.001	0.188 ± 0.000	–

fr. fraction. Ascorbic acid was the positive control, *GAE* gallic acid equivalent

Antioxidant activities of compounds from CE

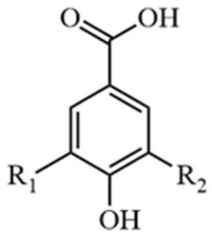
The DPPH and ABTS assays were conducted to evaluate the antioxidant ability of the 13 isolated compounds, and the IC_{50} values were compared with that of ascorbic acid, a representative antioxidant. The results are shown in Table 2. Overall, phenolic acid tended to show higher antioxidant ability than flavonoids. In the DPPH assay, vanillic acid, *p*-coumaric acid, and quercetin demonstrated stronger radical-scavenging activity than ascorbic acid. In particular, vanillic acid was the most effective DPPH radical scavenger from CE. In the ABTS assay, most phenolic acids, quercetin, and luteolin exhibited better radical-scavenging activity than ascorbic acid, and gallic acid and quercetin exhibiting the greatest ability to quench the ABTS radicals (Tables 2, 3).

HPLC–DAD analysis

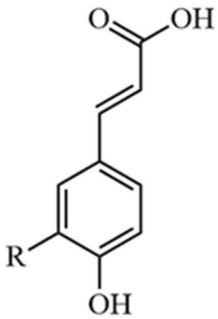
Eight flavonoids and five phenolic acids isolated from CE were analyzed quantitatively using HPLC–DAD. Quantitative analysis was also conducted to compare CE grown in Ganghwa and Hampyeong, which are the two main CE-growing regions. The compound peaks were successfully separated with the corresponding retention times with high resolution (Fig. 2). Good linearity was obtained within the tested concentration range with a correlation coefficient (r^2) of 0.9990–1.0000.

In general, flavonoids are used as marker compounds to evaluate the pharmacological value of plant materials. Flavonoids have been reported to be mainly responsible for biological activity [35]. Quantitative analysis of flavonoids and phenolic acid in CE indicated that their contents varied notably by the region of production (Table 4).

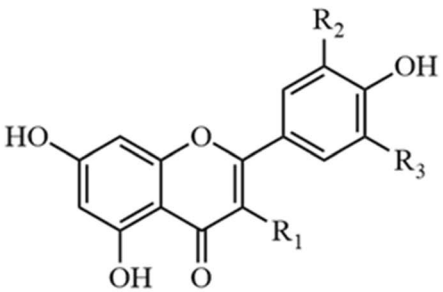
In CE from Hampyeong and Ganghwa, astragalin (4.72 mg/g and 2.85 mg/g, respectively) and tricin (3.00 mg/g and 2.08 mg/g, respectively) were the main flavonoids, and *p*-coumaric acid (4.30 mg/g and 3.39 mg/g, respectively) was the major phenolic acid in the EtOH extract of CE. Overall, the Hampyeong CE had a high phytochemical content, but in terms of ratio, it exhibited a similar pattern to that of Ganghwa CE. The growing environment can be assumed to influence differences in the contents of the active compounds of CE, but it does not change the fundamental constituents (Fig. 3). These findings are consistent with the results of previous studies in which the production of active substances did not differ significantly in the absence of extreme environmental changes [36].



Compound	R_1	R_2
1	OH	OH
2	OH	H
3	OCH_3	H



Compound	R
4	H
6	OCH_3



Compound	R_1	R_2	R_3
5	O-Rut	H	OH
7	O-Glc	H	OH
8	O-Glc	H	H
9	OH	H	OH
10	H	H	OH
11	H	H	H
12	H	OCH_3	OCH_3
13	OH	H	H

Fig. 1 Chemical structures of compounds **1**–**13** of CE collected from Hampyeong

Table 2 DPPH and ABTS radical-scavenging IC_{50} values of compounds **1**–**13** from CE

Compound	IC_{50} (mg/mL)	
	DPPH assay	ABTS assay
Gallic acid (**1**)	0.226 ± 0.001	0.064 ± 0.003
Protocatechuic acid (**2**)	0.411 ± 0.002	0.168 ± 0.007
Vanillic acid (**3**)	0.103 ± 0.000	0.098 ± 0.000
p-Coumaric acid (**4**)	0.146 ± 0.000	0.082 ± 0.000
Rutin (**5**)	0.345 ± 0.001	0.290 ± 0.002
Ferulic acid (**6**)	0.426 ± 0.000	0.131 ± 0.001
Isoquercitrin (**7**)	0.321 ± 0.001	0.291 ± 0.003
Astragalin (**8**)	0.312 ± 0.001	0.188 ± 0.002
Quercetin (**9**)	0.128 ± 0.002	0.068 ± 0.002
Luteolin (**10**)	0.628 ± 0.001	0.152 ± 0.000
Apigenin (**11**)	0.629 ± 0.001	0.263 ± 0.001
Tricin (**12**)	0.558 ± 0.000	0.340 ± 0.010
Kaempferol (**13**)	0.583 ± 0.000	0.340 ± 0.003
Ascorbic acid	0.155 ± 0.000	0.169 ± 0.001

Positive control = Ascorbic acid

Table 3 Calibration curves of phenolic acids and flavonoids

Compound	t_R	Calibration Eq. [a]	Correlation factor, r^{2b}
Gallic acid (**1**)	4.7	Y = 22.267X + 67.033	0.9992
Protocatechuic acid (**2**)	6.6	Y = 25.558X + 76.6	1.0000
Vanillic acid (**3**)	10.2	Y = 32.097X—129.03	0.9992
p-Coumaric acid (**4**)	17.3	Y = 33.68X + 128.23	0.9998
Rutin (**5**)	19.4	Y = 11.017X + 0.2	0.9999
Ferulic acid (**6**)	19.9	Y = 17.545X + 23.808	0.9999
Isoquercitrin (**7**)	22.1	Y = 19.87X + 269.63	0.9991
Astragalin (**8**)	26.2	Y = 23.083X + 73.537	0.9999
Quercetin (**9**)	40.4	Y = 35.876X + 41.054	1.0000
Luteolin (**10**)	41.0	Y = 30.651X—15.65	0.9996
Apigenin (**11**)	43.3	Y = 40.287 X + 72.525	1.0000
Tricin (**12**)	44.2	Y = 12.367 X + 168.19	0.9990
Kaempferol (**13**)	44.9	Y = 35.121X + 292.42	0.9999

t_R retention time

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

[b] r^2 = correlation coefficient for five data points in the calibration curve

In addition, the existing component analysis studies of CE and its related species have provided limited data. Therefore, a component analysis of 13 chemicals isolated from CE was performed for four species (MeOH extracts of *C. difformis, C. microiria, C. sanguinolentus*, and *C. cyperoides*) including CE. Astragalin was not included, but phytochemicals such as quercetin, isoquercitrin, and luteolin, which were isolated from CE, were also included in the four *Cyperus* species extracts (Table 5).

The results of the content analysis showed that the CE extract had the highest content of phenolic acids and flavonoids among the extracts of the five *Cyperus* species (Fig. 4). In the literature, *C. difformis* and several *Cyperus* species have been reported to contain high levels of phenolic compounds, particularly luteolin [37].

Conclusions

Thirteen compounds were identified from the EtOAc and *n*-BuOH fractions of CE. Most of the isolated compounds have been reported to have various biological activities. Among the isolated compounds, astragalin and isoquercitrin were isolated from the genus *Cyperus* for the first time in the present study; similarly, the remaining 11 compounds have not been reported to have been isolated from CE. An HPLC analytical method that can simultaneously analyze 13 compounds isolated from CE was established, and a content comparison of CE by the region of production and a comparative analysis of four related species were conducted. This study demonstrated the academic value of CE, which has not been sufficiently explored. Furthermore, our findings may serve as a reference for using CE as functional material to treat oxidative stresses as well as for expanding our understanding of its antioxidant properties and phytochemical components (Additional file 1: Figs. S1–S26).

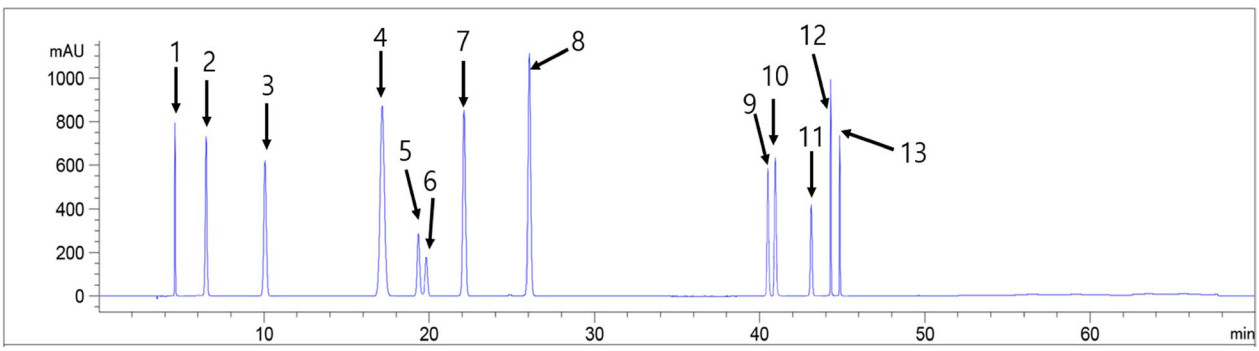


Fig. 2 HPLC chromatogram of compounds **1**–**13** from CE

Table 4 Phenolic acid and flavonoid contents in CE by different regions

Compound	Content (mg/g ext)	
	Hampyeong	Ganghwa
Gallic acid (**1**)	1.09 ± 0.00	0.65 ± 0.01
Protocatechuic acid (**2**)	0.92 ± 0.01	0.36 ± 0.01
Vanillic acid (**3**)	0.94 ± 0.01	0.98 ± 0.00
p-Coumaric acid (**4**)	4.30 ± 0.01	3.39 ± 0.01
Rutin (**5**)	1.02 ± 0.01	0.49 ± 0.01
Ferulic acid (**6**)	0.29 ± 0.00	0.32 ± 0.01
Isoquercitrin (**7**)	2.66 ± 0.01	1.26 ± 0.02
Astragalin (**8**)	4.72 ± 0.00	2.85 ± 0.01
Quercetin (**9**)	0.67 ± 0.01	0.34 ± 0.00
Luteolin (**10**)	1.00 ± 0.01	0.15 ± 0.00
Apigenin (**11**)	0.12 ± 0.00	0.19 ± 0.00
Tricin (**12**)	3.00 ± 0.02	2.08 ± 0.03
Kaempferol (**13**)	1.38 ± 0.01	0.94 ± 0.00

Table 5 Phenolic acid and flavonoid content in *Cyperus* species

Compound	Content (mg/g ext)			
	CD	CM	CS	CC
Gallic acid (**1**)	0.49 ± 0.01	0.04 ± 0.01	0.06 ± 0.01	0.18 ± 0.01
Protocatechuic acid (**2**)	0.51 ± 0.02	0.12 ± 0.00	0.15 ± 0.00	0.26 ± 0.0
Vanillic acid (**3**)	0.93 ± 0.02	0.44 ± 0.01	0.39 ± 0.00	0.63 ± 0.01
p-Coumaric acid (**4**)	0.11 ± 0.01	0.10 ± 0.00	0.06 ± 0.01	trace
Rutin (**5**)	–	–	–	–
Ferulic acid (**6**)	–	–	–	0.38 ± 0.02
Isoquercitrin (**7**)	0.20 ± 0.01	0.09 ± 0.01	0.67 ± 0.01	0.19 ± 0.00
Astragalin (**8**)	–	–	–	–
Quercetin (**9**)	0.31 ± 0.02	–	–	–
Luteolin (**10**)	0.45 ± 0.03	0.27 ± 0.00	1.85 ± 0.04	0.39 ± 0.01
Apigenin (**11**)	–	–	–	–
Tricin (**12**)	Trace	Trace	Trace	Trace
Kaempferol (**13**)	–	Trace	Trace	–

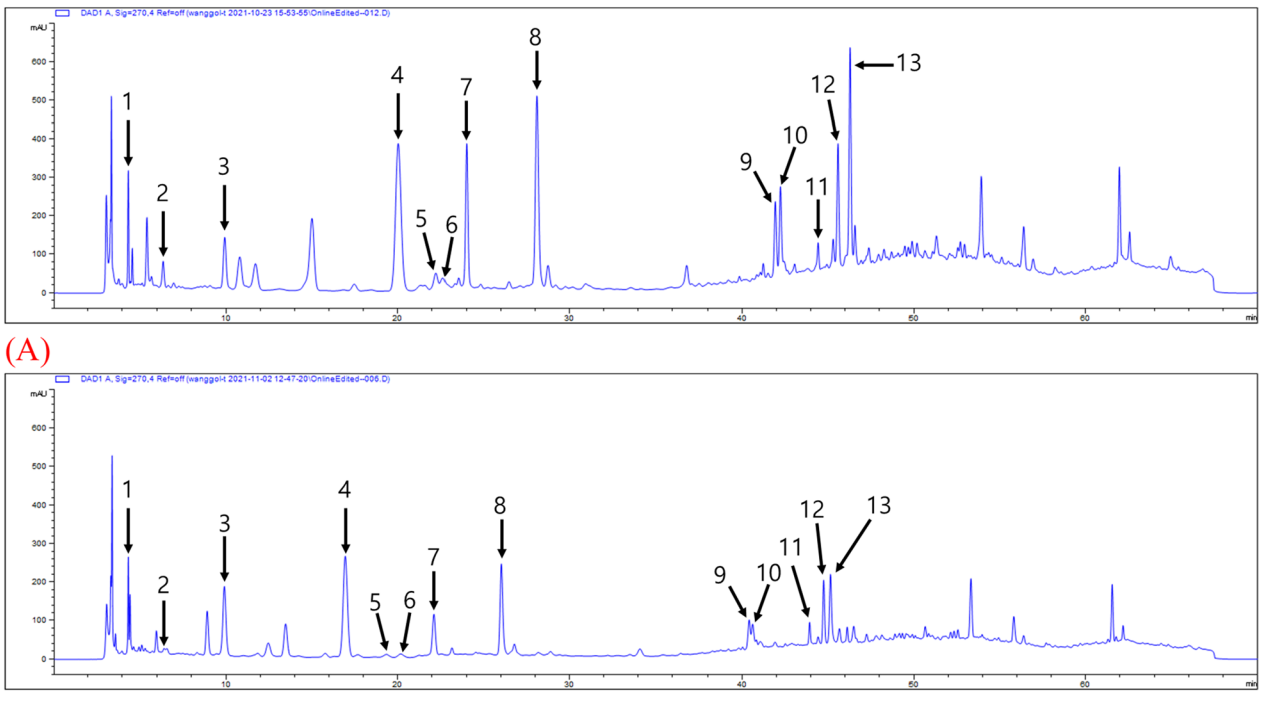
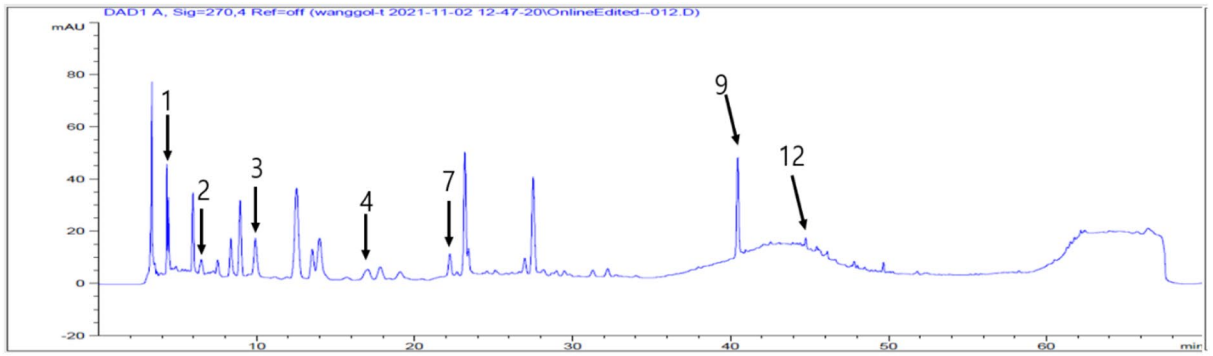
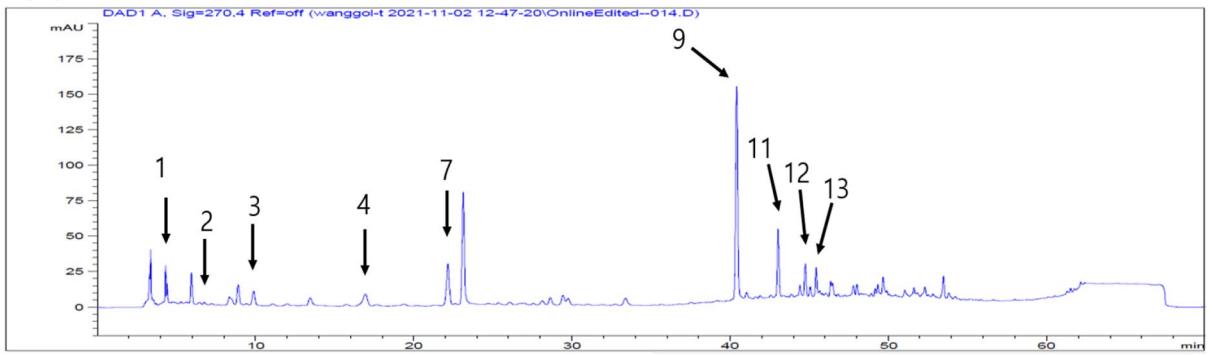


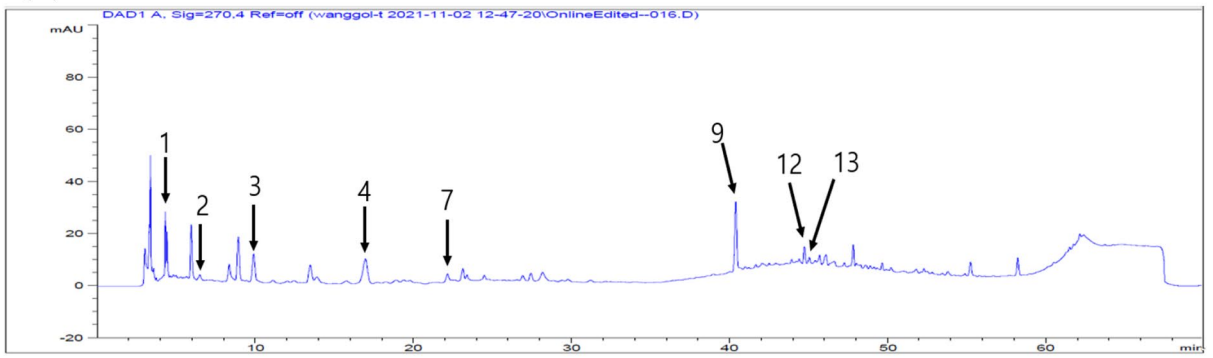
Fig. 3 HPLC chromatograms of CE collected from Hampyeong **A** and Ganghwa **B**



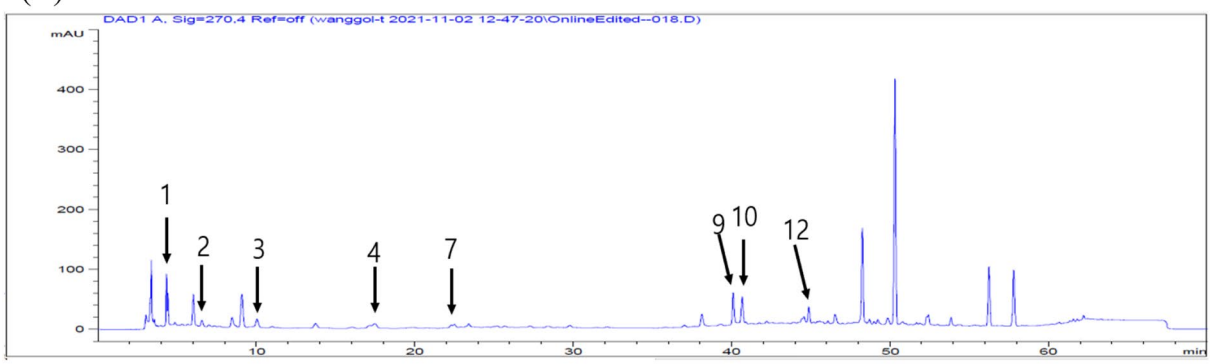
(A)



(B)



(C)



(D)

Fig. 4 HPLC chromatograms of CD **A**, CM **B**, CS **C**, and CC **D**

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13765-023-00820-3.

Additional file 1: Fig. S1. 1H-NMR spectrum of compound **1** (Gallic acid). **Fig. S2.** 13C-NMR spectrum of compound **1** (Gallic acid). **Fig. S3.** 1H-NMR spectrum of compound 2 (Protocatechuic acid). **Fig. S4.** 13C-NMR spectrum of compound **2** (Protocatechuic acid). **Fig. S5.** 1H-NMR spectrum of compound **3** (Vanillic acid). **Fig. S6.** 13C-NMR spectrum of compound **3** (Vanillic acid). **Fig. S7.** 1H-NMR spectrum of compound **4** (P-coumaric acid). **Fig. S8.** 13C-NMR spectrum of compound **4** (P-coumaric acid). **Fig. S9.** 1H-NMR spectrum of compound **5** (Rutin). **Fig. S10.** 13C-NMR spectrum of compound **5** (Rutin). **Fig. S11.** 1H-NMR spectrum of compound **6** (Ferulic acid). **Fig. S12.** 13C-NMR spectrum of compound **6** (Ferulic acid). **Fig. S13.** 1H-NMR spectrum of compound **7** (Isoquercetin). **Fig. S14.** 13C-NMR spectrum of compound **7** (Isoquercetin). **Fig. S15.** 1H-NMR spectrum of compound **8** (Astragalin). **Fig. S16.** 13C-NMR spectrum of compound **8** (Astragalin). **Fig. S17.** 1H-NMR spectrum of compound 9 (Quercetin). **Fig. S18.** 13C-NMR spectrum of compound **9** (Quercetin). **Fig. S19.** 1H-NMR spectrum of compound **10** (Luteolin). **Fig. S20.** 13C-NMR spectrum of compound **10** (Luteolin). **Fig. S21.** 1H-NMR spectrum of **compound 11** (Apigenin). **Fig. S22.** 13C-NMR spectrum of compound **11** (Apigenin). **Fig. S23.** 1H-NMR spectrum of compound **12** (Tricin). **Fig. S24.** 13C-NMR spectrum of compound **12** (Tricin). **Fig. S25.** 1H-NMR spectrum of compound **13** (Kaempferol). **Fig. S26.** 13C-NMR spectrum of compound **13** (Kaempferol).

Acknowledgements
Not applicable.

Author contributions
JC: isolation of phenolic compounds, HDL: structure elucidation of phenolic compounds, HC: anti-oxidant activity measurement, C-DL: HPLC analysis of phenolic compounds, GHT and HK: data curation and draft writing, SKM: draft writing, SL: experimental design and writing.

Funding
This research was supported by grants from the Natural Product Institute of Science and Technology (2021), Anseong, Korea and the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2018R1A6A1A03025159).

Availability of data and materials
Not applicable.

Declarations

Competing interests
On behalf of all authors, the corresponding author states that there is no competing interests.

Received: 8 July 2023 Accepted: 3 September 2023
Published online: 20 September 2023

References

1. Lee J-R, Kim C-S, Lee I-Y (2011) *Cyperus esculentus* L. —a new weed in Korea. Korean J Weed Sci 31:313–318
2. Kim K-I, Kim K-U, Shin D-H, Lee I-J (1998) Growth characteristics of purple nutsedge (*Cyperus rotundus* L.) and establishment of its effective control method Korean. J Weed Sci 18:136–145
3. Bezerra JJL, Pinheiro AAV (2022) Traditional uses, phytochemistry, and anticancer potential of *Cyperus rotundus* L. (Cyperaceae): a systematic review South African. J Bot 144:175–186
4. Li J (2008) Flora of China Harv Pap Bot. Higher Education Press, Beijing
5. Burkill HM (1995) The useful plants of west tropical Africa, the useful plants of west tropical Africa. Royal Botanic Gardens, Kew
6. Cerutti PA (1991) Oxidant stress and carcinogenesis. Eur J Clin Invest 21:1–5
7. Fridovich I (1978) The biology of oxygen radicals. Science 201:875–880
8. Suzuki YJ, Forman HJ, Sevanian A (1997) Oxidants as stimulators of signal transduction. Free Radic Biol Med 22:269–285
9. Vuotto ML, Basile A, Moscatiello V, De Sole P, Castaldo-Cobianchi R, Laghi E, Ielpo MTL (2000) Antimicrobial and antioxidant activities of *Feijoa sellowiana* fruit. Int J Antimicrob Agents 13:197–201
10. Moskovitz J, Bin Yim M, Chock PB (2002) Free radicals and disease. Arch Biochem Biophys 397:354–359
11. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J (2007) Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 39:44–84
12. Singh PN, Singh SB (1980) A new saponin from mature tubers of *Cyperus rotundus*. Phytochemistry 19:2056
13. Himaja M, Ranjitha A, Ramana MV, Anand M, Asif K (2010) Phytochemical screening and antioxidant activity of rhizome part of *Curcuma zedoaria*. Int J Res Ayurveda Pharm 1:414–417
14. Broadhurst CL, Polansky MM, Anderson RA (2000) Insulin-like biological activity of culinary and medicinal plant aqueous extracts in vitro. J Agric Food Chem 48:849–852
15. Yang JH, Lin HC, Mau JL (2002) Antioxidant properties of several commercial mushrooms. Food Chem 77:229–235
16. Pandurangan A, Khosa RL, Hemalatha S (2008) Evaluation of anti-inflammatory and analgesic activity of root extract of *Solanum trilobatum* Linn. Iran J Pharm Res 7:217–221
17. Soumaya KJ, Dhekra M, Fadwa C, Zied G, Ilef L, Kamel G, Leila CG (2013) Pharmacological, antioxidant, genotoxic studies and modulation of rat splenocyte functions by *Cyperus rotundus* extracts. BMC Complement Altern Med 13:1–11
18. Lu Y, Yeap Foo L (2000) Antioxidant and radical scavenging activities of polyphenols from apple pomace. Food Chem 68:81–85
19. Choi Y, Kim M, Shin J-J, Park J-M, Lee J (2003) The antioxidant activities of the some commercial teas. J Korean Soc Food Sci Nutr 32:723–727
20. Heo J-C, Park J-Y, An S-M, Lee J-M, Yun C-Y, Shin H-M, Kwon T-K, Lee S-H (2006) Anti-oxidant and anti-tumor activities of crude extracts by *Gastrodia elata* Blume. Korean J Food Preserv 13:83–87
21. Dewanto V, Wu X, Liu RH (2002) Processed sweet corn has higher antioxidant activity. J Agric Food Chem 50:4959–4964
22. López-Martínez LM, Santacruz-Ortega H, Navarro RE, Sotelo-Mundo RR, González-Aguilar GA (2015) A 1H NMR investigation of the interaction between phenolic acids found in mango (*Manguifera indica* cv Ataulfo) and Papaya (*Carica papaya* cv *Maradol*) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals. PLoS One 10:0140242
23. Kwak JH, Kim HJ, Lee KH, Kang SC, Zee OP (2009) Antioxidative iridoid glycosides and phenolic compounds from *Veronica peregrina*. Arch Pharmacal Res 32:207–213
24. Chang SW, Kim KH, Lee IK, Choi SU, Ryu SY, Lee KR (2009) Phytochemical constituents of *Bistorta manshuriensis*. Nat Prod Sci 15:234–240
25. Świsłocka R, Kowczyk-Sadowy M, Kalinowska M, Lewandowski W (2012) Spectroscopic (FT-IR, FT-Raman, ^{1}H and ^{13}C NMR) and theoretical studies of *p*-coumaric acid and alkali metal *p*-coumarates. Spectroscopy 27:35–48
26. Fathiazad F, Delazar A, Amiri R, Sarker SD (2010) Extraction of flavonoids and quantification of rutin from waste tobacco leaves. Iran J Pharm Res 5:222–227
27. Kalinowska M, Piekut J, Bruss A, Follet C, Sienkiewicz-Gromiuk J, Świsłocka R, Rzączyńska Z, Lewandowski W, (2014) Spectroscopic (FT-IR, FT-Raman, ^{1}H, ^{13}C NMR, UV/VIS), thermogravimetric and antimicrobial studies of Ca(II), Mn(II), Cu(II), Zn(II) and Cd(II) complexes of ferulic acid. Spectrochim Acta Part A Mol Biomol Spectrosc 122:631–638
28. Salem JH, Humeau C, Chevalot I, Harscoat-Schiavo C, Vanderesse R, Blanchard F, Fick M (2010) Effect of acyl donor chain length on isoquercitrin acylation and biological activities of corresponding esters. Process Biochem 45:382–389
29. Rezende FM, Ferreira MJP, Clausen MH, Rossi M, Furlan CM (2019) Acylated flavonoid glycosides are the main pigments that determine the flower colour of the brazilian native tree *Tibouchina pulchra* (Cham) Cogn. Mol 24:718

30. Lallemand JY, Duteil M (1977) ^{13}C NMR spectra of quercetin and rutin. Org Magn Reson 9:179–180
31. Jo HW, Park JC (2008) Phenolic compounds isolated from the leaves of *Angelica keiskei* showing DPPH radical scavenging effect. Korean J Pharmacogn 39:146–149
32. Lee JW, Kang SC, Bae JJ, Lee KB, Kwak JH (2015) Flavonoids from the flower of *Clerodendrum trichotomum*. Korean J Pharmacogn 46:289–294
33. Woo HS, Lee SY, Hwang BS, Jeong SC, Kim DW (2017) Isolation and quantitative analysis of tricin from ears of *Phragmites communis*. Korean J Pharmacogn 48:77–81
34. De Souza LA, Tavares WMG, Lopes APM, Soeiro MM, De Almeida WB (2017) Structural analysis of flavonoids in solution through DFT ^{1}H NMR chemical shift calculations: Epigallocatechin, kaempferol and quercetin. Chem Phys Lett 676:46–52
35. Lee SH, Kim HW, Lee MK, Kim YJ, Asamenew G, Cha YS, Kim JB (2018) Phenolic profiling and quantitative determination of common sage (*Salvia plebeia* R. Br.) by UPLC-DAD-QTOF/MS. Eur Food Res Technol 244:1637–1646
36. Yang L, Wen KS, Ruan X, Zhao YX, Wei F, Wang Q (2018) Response of plant secondary metabolites to environmental factors. Mol 23:762
37. Sayed HM, Mohamed MH, Farag SF, Mohamed GA (2001) Phytochemical and biological investigations of *Cyperus rotundus* L. Bull Fac Pharm 39:195–203

Publisher's Note