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Research Article

Antioxidant, Anticancer, and Neuroprotective Activities and Phytochemical Analysis of Germinated Shoots

Juree Kim,[1] Chung Ho Choi,[2] Ah Young Lee,[3] and Sanghyun Lee[1,4]

[1]*Department of Plant Science and Technology, Chung-Ang University, Anseong 17546, Republic of Korea*
[2]*Gyeonggi-do Forestry Environment Research Center, Osan 18118, Republic of Korea*
[3]*Department of Food Science and Nutrition, Gyeongsang National University, Jinju 52828, Republic of Korea*
[4]*Natural Product Institute of Science and Technology, Anseong 17546, Republic of Korea*

Correspondence should be addressed to Ah Young Lee; aylee@gnu.ac.kr and Sanghyun Lee; slee@cau.ac.kr

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This study evaluated the antioxidant, anticancer, and neuroprotective activities of germinated shoots (cotyledon and true leaves) of wild plants and measured their total phenol, flavonoid, quercetin, and vitamin C contents. The ethanol extract of *Geum aleppicum* (GA) showed high DPPH and ABTS radical scavenging activities. GA treatment also significantly increased cell viability against hydrogen peroxide-induced oxidative stress in SH-SY5Y neuronal cells, indicating that GA had antioxidant and neuroprotective properties. In AGS gastric cancer cells, the cotyledon of *Pinus densiflora* (PD1) and the true leaves of *Chamaecyparis obtusa* (CHA2) significantly inhibited cell proliferation, showing that PD1 and CHA2 elicited anticancer effect. The total phenol content was the highest in the shoots of GA, and the total flavonoid content was the highest in the shoots of true leaves of *P. densiflora*. The quercetin and vitamin C contents were the highest in the CHA2 and the cotyledon of GA, respectively. In conclusion, this study suggested the antioxidant, anticancer, and neuroprotective activities of the germinated shoots of wild plants and their high total phenol, flavonoid, quercetin, and vitamin C contents. These findings were noteworthy in the case of the germinated shoot of GA and provided a basis for studying functional forestry income resources. *Practical Applications*. Our results demonstrated that GA exerts strong DPPH and ABTS radical scavenging activities. It also plays a neuroprotective role in oxidative stress-induced cell damage. These effects can be linked to their polyphenol and flavonoid contents in the extract. Moreover, PD1 treatment has inhibitory effect on gastric tumor cells growth and exhibited an effective •OH radical scavenging property. These findings indicated that germinated shoot extracts have potential for development of functional food with therapeutic applications.

1. Introduction

Synthetic medicines have widely been used to treat many diseases. Despite the benefits of synthetic drugs or antioxidants, unintended side effects or certain toxic effects on human health have been reported. From this point of view, studies have increasingly focused on natural sources such as sprouts, which are more economical and less toxic than synthetic drugs [1]. Studies have also revealed that the intake of herbal medicine from plants is related to a reduced risk of chronic diseases [2, 3]. Vitamins, phenolic compounds, and flavonoids in plants have health benefits, including antioxidant, anticancer, and anti-inflammatory properties. The nutritional and medicinal value of edible seeds can be enhanced during germination that leads to the accumulation of secondary metabolites and bioactive compounds with antioxidant, antidiabetic, and anticancer effects [4, 5]. Although germinated shoots have been applied to improve their phytochemical composition, scientific evidence supporting their biological activities has not been fully investigated; thus, their antioxidant, anticancer, and neuroprotective effects should be evaluated to develop functional foods as new promising natural sources.

Sprouts have been increasingly consumed because of the influence of well-being, and they have been considered healthy and functional foods rather than vegetables. For

example, buckwheat sprouts and barley seedlings are effective in improving diabetes and various vascular diseases, such as high blood pressure [6–8]. Broccoli sprouts are effective in improving obesity and eye health [9, 10]. Turnip sprouts are effective in lowering high blood pressure [11]. The fiber and vitamin C contents of Chinese cabbage sprouts, which are good for constipation, increase significantly compared with those of seeds.

The size of domestic markets using plant sprouts had been steadily increasing to KRW 95.6 billion until 2015, with an average annual growth rate of 17.1%. In United States, wheat sprouts have been used as healthy foods since the 1930s; in England, sprouts such as radish, mustard, and Chinese cabbage have been used as food since the time of Queen Victoria. In the Heian period, blue juices such as rice and barley sprouts were used in Japan. The market size of sprouted vegetable seeds in Korea is estimated to be about KRW 5 billion, and approximately 1,000 tons or more of seeds are used. Furthermore, approximately 80% of the country is dependent on imports. Commonly produced and consumed sprouts are agricultural crops such as radish, Chinese cabbage, and barley; other sprouts include some forest plants.

In this study, we determined the antioxidant, anticancer, and neuroprotective effects of the germinated shoots of wild plants and their total phenol, flavonoid, quercetin, and vitamin C contents for the development and utilization of forestry income resources.

2. Materials and Methods

2.1. Plant Materials. The cotyledon and true leaves of eight wild plant species were used (Table 1): cotyledon of *Achyranthes bidentata* (AB1), true leaves of *A. bidentata* (AB2), cotyledon of *Securinega suffruticosa* (SS1), true leaves of *S. suffruticosa* (SS2), cotyledon of *Chamaecyparis obtusa* (CHA1), true leaves of *C. obtusa* (CHA2), cotyledon of *Allium dumebuchum* (AD1), true leaves of *A. dumebuchum* (AD2), cotyledon of *Geum aleppicum* (GA1), true leaves of *G. aleppicum* (GA2), cotyledon of *Cryptotaenia japonica* (CJ1), true leaves of *C. japonica* (CJ2), cotyledon of *Ulmus pumila* (UP1), true leaves of *U. pumila* (UP2), cotyledon of *Pinus densiflora* (PD1), and true leaves of *P. densiflora* (PD2). A voucher specimen was deposited at the herbarium of Department of Plant Science and Technology, Chung-Ang University, Anseong, Korea.

2.2. Shoot Germination. Dried germinated shoots (cotyledon and true leaves) were provided by Gyeonggi-do Forestry Environment Research Center (Osan, Korea). Sowing was performed in a horticultural medium (Nongwoo Bio Co. Ltd., Suwon, Korea) in early April 2021 to obtain sprout samples. Among the test materials, dormant seeds were sown after they were prechilled for 30 days to break dormancy, and the remaining nondormant seeds were immersed in distilled water for 1 day before sowing. The germinated individuals were separated into cotyledons and true leaves (Figure 1). Then, they were harvested, washed, and used for the subsequent experiments. The germination characteristics of seeds used for experiment are shown in Table 2.

2.3. Instruments, Chemicals, and Reagents. Ethanol (EtOH; Samchun Pure Chemicals, Pyeongtaek, Korea) was used for sample extraction. Standard compounds, namely, tannic acid, quercetin, and vitamin C, were obtained from Natural Product Institute of Science and Technology (https://www.nist.re.kr/), Anseong 17546, Korea. Folin–Ciocalteu reagent, Na_2CO_3, and $AlCl_3$ were purchased from Sigma-Aldrich (MA, USA). Total phenol and flavonoid contents were measured using a microplate reader (Epoch Microplate Spectrophotometer, BioTek, Winooski, USA). HPLC-grade water and acetonitrile were bought from J. T. Baker (Phillipsburg, PA, USA). Quercetin and vitamin C were analyzed using an HPLC system (Waters Alliance e2695 Separations Module, USA) equipped with a pump, a detector (Waters 996 photodiode array detector, USA), an auto sampler, and a column oven.

2.4. Sample Extraction. The dried germinated shoots (8 g each) were cut into 5–7 cm lengths, grinded, and extracted with EtOH (160 mL each) under reflux thrice for 3 h. The samples were filtered and evaporated using a rotary evaporator to obtain the extracts.

2.5. Total Phenol Content Measurement. The total phenol content of the extract was measured by modifying the Folin–Ciocalteu method [12]. Tannic acid was dissolved in water, diluted to serial concentrations (7.8125–125 μg/mL), and used to make the calibration curve. Sample solutions were prepared by dissolving the extract in water (1 mg/mL), and 60 μL of the solution was added to a 96-well plate. Then, 40 μL of 2N Folin–Ciocalteu reagent was added to the solution, and 100 μL of 7.5% Na_2CO_3 was added to the mixture. After being mixed using a micro mixer (Mx4, Finepcr, Gunpo, Korea), the samples were incubated in a dark place at room temperature for 30 min. Absorbance was measured at 760 nm by using a microplate reader (Epoch Microplate Spectrophotometer, BioTek, Winooski, USA). The total phenol content was measured in tannic acid equivalents (mg TAE/g extract).

2.6. Total Flavonoid Content Measurement. The total flavonoid content of the extract was measured by modifying the aluminum chloride method [13]. Quercetin was dissolved in 80% EtOH, diluted to serial concentrations (7.8125-125 μg/mL), and used to make the calibration curve. Sample solutions were prepared by dissolving the extract in 80% EtOH (1 mg/mL). Then, 100 μL of the sample solution was added to a 96-well plate, and 100 μL of 2% $AlCl_3$ was added to the solution. The solution was mixed using the micro mixer and incubated in a dark place at room temperature for 10 min. Absorbance was measured at 430 nm by using a microplate reader. The total flavonoid content was measured in quercetin equivalents (mg QE/g extract).

Table 1: Source of plant materials.

Plant species (family name)	Collection location
A. bidentata Blume var. *japonica* Miq. (Amaranthaceae)	Choji-ri, Gilsang-myeon, Ganghwa-gun
S. suffruticosa (Pall.) Rehder (Euphorbiaceae)	Joan-ri, Joan-myeon, Namyangju-si
C. obtusa (Siebold & Zucc.) Endl. (Cupressaceae)	Chobu-ri, Mohyeon-eup, Cheoin-gu, Yongin-si
A. dumebuchum H. J. Choi (Liliaceae)	Maeryong-dong, Yeoju-si
G. aleppicum Jacq. (Rosaceae)	Gurae-ri, Sangdong-eup, Yeongwol-gun
C. japonica Hassk. (Apiaceae)	Sucheong-don, Osan-si
U. pumila L. (Ulmaceae)	Nakcheon-ri, Imgye-myeon, Jeongseon-gun
P. densiflora Siebold & Zucc. (Pinaceae)	Jungjang-ri, Anmyeon-eup, Taean-gun

(a) (b)

Figure 1: Germinated shoots of (a) cotyledon of *Geum aleppicum* (GA1) and (b) true leaves of *G. aleppicum* (GA2).

Table 2: The germination characteristics of seeds used for experiment.

Plant species	[z]PG (%)	MGT (day)	Remark
A. bidentata Blume var. *japonica* Miq.	74.0 ± 2.0	11.5 ± 1.4	—
S. suffruticosa (Pall.) Rehder	79.3 ± 5.0	14.7 ± 0.3	Prechilling 30 days
C. obtusa (Siebold & Zucc.) Endl.	32.7 ± 3.2	32.5 ± 0.1	—
A. dumebuchum H. J. Choi	82.7 ± 1.2	13.2 ± 0.3	—
G. aleppicum Jacq.	97.5 ± 2.5	3.3 ± 0.1	—
C. japonica Hassk.	89.3 ± 1.2	49.0 ± 1.8	—
U. pumila L.	72.7 ± 3.1	12.2 ± 0.2	—
P. densiflora Siebold & Zucc.	84.7 ± 4.6	37.1 ± 2.9	—

[z]PG, percent germination; MGT, mean germination time.

2.7. Preparation of Stock Solutions for HPLC Analysis. For quercetin analysis, the extracts of the geminated shoots (20 mg/mL each) were dissolved in methanol (MeOH); for vitamin C analysis, the extracts were prepared by dissolving it at a concentration of 4 mg/mL. Quercetin and ascorbic acid (1 mg/mL each; standard compounds) were also dissolved in MeOH. All sample and standard stock solutions were prepared by sonicating for 20 min and filtering through a 0.45 μm polyvinylidene difluoride membrane filter.

2.8. Antioxidant Activity. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was evaluated in accordance with previously described methods [14] with some modifications. In brief, 100 μL of each sample (100 μg/mL) was added to 100 μL of 300 μM DPPH solution in a 96-well microplate. After 30 min of reaction in the dark at room temperature, the absorbance of each well was read at 540 nm. DPPH radical scavenging activity was expressed as percentage and was calculated according to the following equation: DPPH radical scavenging activity (%) = $[1 - (\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}}/\text{Abs}_{\text{control}})] \times 100$. $\text{Abs}_{\text{sample}}$, absorbance of the sample; $\text{Abs}_{\text{blank}}$, absorbance of the blank; $\text{Abs}_{\text{control}}$, absorbance of the control.

2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity was assessed in accordance with the procedure of Re et al. [15]. The ABTS solution was prepared by mixing 7.4 mM ABTS with 2.6 mM potassium persulfate in the dark at room temperature for 6 h before use. This solution was further diluted to an absorbance of 0.8-0.9 at 600 nm. For the assay, 100 μL of each sample (100 μg/mL) was mixed with 100 μL of ABTS solution in a 96-well microplate. After 6 min, the absorbance of each well was read at 600 nm. ABTS radical scavenging activity was expressed as percentage and was calculated according to the following equation: ABTS radical

scavenging activity (%) = $[1 - (Abs_{sample} - Abs_{blank}/Abs_{control})] \times 100$. Abs_{sample}, absorbance of the sample; Abs_{blank}, absorbance of the blank; $Abs_{control}$, absorbance of the control.

Hydroxyl radical (•OH) scavenging activity was evaluated in accordance with the method of Chung et al. [16]. The samples (100 μg/mL) were added to the reaction mixture containing 10 mM H_2O_2 and 10 mM $FeSO_4$·H_2O-EDTA with 10 mM 2-deoxyribose solution and incubated at 37°C for 4 h. After incubation, 1.0% thiobarbituric acid and 2.8% trichloroacetic acid were added to the mixture and boiled at 100°C for 20 min. •OH radical scavenging ability was measured at 490 nm. •OH radical scavenging activity was expressed as percentage and was calculated according to the following equation: •OH radical scavenging activity (%) = $[1 - (Abs_{sample} - Abs_{blank}/Abs_{control})] \times 100$. Abs_{sample}, absorbance of the sample; Abs_{blank}, absorbance of the blank; $Abs_{control}$, absorbance of the control.

2.9. Anticancer Effect. AGS human gastric adenocarcinoma cells (Korea Cell Line Bank, KCLB) were cultured in Roswell Park Memorial Institute Medium-1640 (RPMI-1640) with 100 U/mL of penicillin/streptomycin and 10% fetal bovine serum (FBS) in a 5% CO_2 incubator at 37°C. They were seeded in 24-well microplates at a density of 5×10^4 cells·mL^{-1} overnight. On next day, they were treated with the samples (100 μg/mL) for 48 h, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (0.5 mg/mL) was treated in a CO_2 incubator at 37°C for 4 h. After the MTT-containing medium was removed, formazan was dissolved in 1 mL of dimethyl sulfoxide (DMSO) and quantified by measuring the absorbance at 540 nm.

2.10. Neuroprotective Effect. SH-SY5Y human neuroblastoma cells (KCLB) were cultured in DMEM containing 100 U/mL of penicillin/streptomycin and 10% FBS in a 5% CO_2 incubator at 37°C. The cells were seeded (5×104 cells/mL) in 96-well microplates overnight to evaluate cell viability. The cells were initially treated with the samples (100 μg/mL) for 2 h and then with H_2O_2 (250 μM) for 24 h to induce oxidative stress. MTT solution (0.5 mg/mL) was added to each well and further incubated in a 5% CO_2 incubator at 37°C for 4 h. Formazan crystals were dissolved in 1 mL of DMSO, and formazan was quantified by measuring the absorbance at 540 nm.

2.11. HPLC Conditions. Quercetin and vitamin C were quantitatively analyzed through HPLC by using reverse-phase INNO C18 column (4.6×250 mm, 5 μm). The elution system was gradient, and the mobile phase was composed of water (A) and acetonitrile (B). The injection volume and flow rate were 10 μL and 1 mL/min, respectively. In quercetin analysis, elution was performed under the following parameters: 95% A for 0 min, 75% A for 25 min, 40% B for 30 min, 100% B for 35 min, 100% B for 40 min, 95% A for 45 min, and 95% A for 55 min. Quercetin was detected at a wavelength of 254 nm, and the column temperature was 30°C. In vitamin C analysis, elution was conducted under the following parameters: 75% A for 0 min, 75% A for 5 min, 100% B for 23 min, 25% B for 27 min, and 25% B for 35 min. UV was set at 245 nm, and the column temperature was 20°C.

2.12. Calibration Curves. Working solutions were prepared by diluting the standard stock solutions to different serial concentrations. The calibration curves of the standards were constructed by plotting the peak area (Y) against the concentration (X, μg/mL). All values were presented as mean ± standard deviation (SD; $n = 3$).

2.13. Statistical Analysis. Data were represented as mean ± SD and analyzed through ANOVA followed by Duncan's post hoc test by using SPSS Statistics (ver. 19.0, SPSS, Inc., Chicago, IL, USA) to compare the means across multiple groups at a significance level of $p < 0.05$.

3. Results and Discussion

3.1. Antioxidant Activities of Shoot Extracts. Free radicals, including reactive oxygen species and reactive nitrogen species, are normal biomolecules generated through aerobic metabolism [17]. However, the excessive generation of free radicals in the body can cause DNA, protein, and lipid oxidation, leading to cell death and tissue injury [18]. Free radical-mediated damage contributes to chronic health problems, such as cancer, diabetes, obesity, cardiovascular diseases, and neurodegenerative diseases [19–21]. Dietary antioxidants likely inhibit oxidation [22]. However, synthetic antioxidants, such as butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT) have potential toxicological and side effects on human health [23, 24]. In this sense, many natural plants, which contain polyphenols, flavonoids, and carotenoids, are better than chemical compounds; thus, natural sources with antioxidant property should be explored. The antioxidant properties of the germinated shoot extract were evaluated via a DPPH, ABTS, and •OH radical scavenging assay (Table 3). As a result, GA1 had a strong DPPH radical scavenging activity of 40.26%. Moreover, PD1 showed the second-highest radical scavenging ability (32.15%). Among the 16 kinds of germinated shoot extracts, GA1 and GA2 could efficiently scavenge the ABTS radical. Moreover, all extracts exhibited strong •OH radical scavenging effects (above 80%). In particular, the •OH radical scavenging ability of PD1 was the highest (95.91%).

3.2. Neuroprotective Effect of Shoot Extracts. Oxidative stress is one of the major causes of neurodegenerative diseases, such as Alzheimer's disease. H_2O_2 is commonly used as an inducer of oxidative damage; thus, a H_2O_2-induced neuronal cell model can be suitable for studying neurodegenerative disorders. In this study, cell viability was determined via the MTT assay to investigate whether the germinated shoot extracts elicited a neuroprotective effect against H_2O_2 in

Table 3: Antioxidant effect of germinated shoot extracts.

Sample (100 μg/mL)	Radical scavenging activity (%)		
	DPPH	ABTS	•OH
Cotyledon of *Achyranthes bidentata* (AB1)	26.15 ± 0.83^{d}	23.47 ± 0.68^{fg}	82.82 ± 0.18^{g}
True leaves of *Achyranthes bidentata* (AB2)	16.30 ± 1.75^{f}	22.36 ± 0.98^{g}	88.13 ± 0.50^{ef}
Cotyledon of *Securinega suffruticosa* (SS1)	17.94 ± 1.60^{f}	28.88 ± 0.67^{e}	83.38 ± 0.41^{g}
True leaves of *Securinega suffruticosa* (SS2)	25.58 ± 1.14^{d}	75.97 ± 1.47^{b}	88.71 ± 1.58^{ef}
Cotyledon of *Chamaecyparis obtusa* (CHA1)	21.29 ± 1.68^{f}	19.30 ± 0.71^{h}	94.63 ± 0.47^{ab}
True leaves of *Chamaecyparis obtusa* (CHA2)	20.96 ± 1.54^{f}	11.49 ± 1.80^{i}	91.42 ± 0.37^{d}
Cotyledon of *Allium dumebuchum* (AD1)	1.77 ± 1.16^{i}	12.49 ± 0.43^{i}	87.39 ± 0.51^{f}
True leaves of *Allium dumebuchum* (AD2)	4.24 ± 0.86^{h}	28.98 ± 1.60^{e}	92.39 ± 0.43^{cd}
Cotyledon of *Geum aleppicum* (GA1)	40.26 ± 1.62^{a}	99.88 ± 0.43^{a}	89.46 ± 1.88^{e}
True leaves of *Geum aleppicum* (GA2)	29.25 ± 1.80^{c}	100.34 ± 0.39^{a}	88.11 ± 1.29^{ef}
Cotyledon of *Cryptotaenia japonica* (CJ1)	5.32 ± 2.16^{h}	24.33 ± 0.29^{f}	94.16 ± 0.37^{bc}
True leaves of *Cryptotaenia japonica* (CJ2)	3.47 ± 0.44^{hi}	70.00 ± 4.86^{c}	92.75 ± 0.13^{cd}
Cotyledon of *Ulmus pumila* (UP1)	21.58 ± 2.93^{e}	13.16 ± 1.04^{i}	93.48 ± 2.30^{bc}
True leaves of *Ulmus pumila* (UP2)	21.78 ± 0.85^{e}	47.76 ± 0.87^{d}	93.05 ± 1.56^{bcd}
Cotyledon of *Pinus densiflora* (PD1)	32.15 ± 2.15^{b}	1.77 ± 1.72^{j}	95.91 ± 2.98^{a}
True leaves of *Pinus densiflora* (PD2)	12.99 ± 1.64^{g}	28.95 ± 0.65^{e}	93.91 ± 2.28^{bc}

Values are expressed as mean ± SD. $^{a-j}$Means with different letters indicate significant differences ($p < 0.05$) by Duncan's multiple range test.

neuronal cells (Figure 2). H_2O_2 treatment caused a significant cytotoxic effect on SH-SY5Y cells, showing 45.08% of cell viability, compared to nontreated normal group (100%). However, pretreatment with GA1 and GA2 significantly increased cell viability by 55.74% and 56.63%, respectively. Our findings suggested that GA1 and GA2 could protect neuronal cells against H_2O_2-induced oxidative damage.

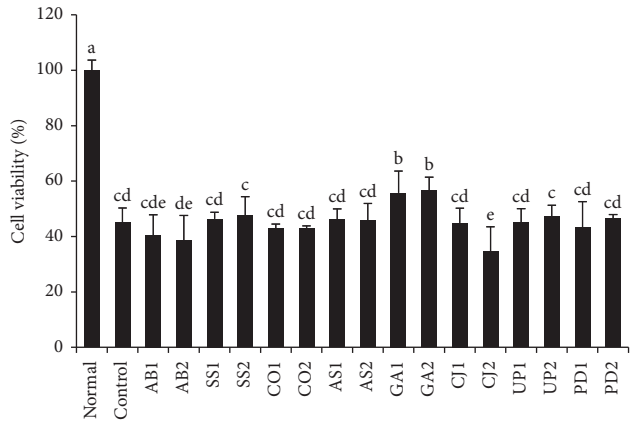

Figure 2: Neuroprotective effect of the EtOH extracts of germinated shoots on cell viability in H_2O_2-treated SH-SY5Y neuronal cells. Values are expressed as mean ± SD. $^{a-e}$Means with different letters indicate significant differences ($p < 0.05$) by Duncan's multiple range test. Normal, nontreated group; control, H_2O_2-treated group.

3.3. Total Phenol and Flavonoid Contents of Shoot Extracts. The total phenol and flavonoid contents in the extracts of the germinated shoots were measured by modifying the Folin–Ciocalteu and aluminum chloride methods, respectively (Table 4). The total phenol content was expressed as tannic acid equivalents in milligrams per gram of extracts. It was the highest in the extracts of GA2, followed by GA1 (84.05 and 78.88 mg·TAE/g, respectively). The range of the total phenol content was 12.75–84.05 mg·TAE/g extract. Generally, the total phenol content of the true leaves and the cotyledon was similar, or the true leaves had a higher content. Plants rich in polyphenols have various biological activities, such as antioxidant, antiviral, antimicrobial, and antidiabetic effects [25–27]. In addition, polyphenolic compounds from fruits or vegetables have inhibitory effects on carcinogenesis and mutagenesis when they are consumed by up to 1 g daily [28].

GA is a perennial flowering plant in Rosaceae, native to Europe, Asia, and North America. It is commonly called common avens or yellow avens [29]. GA has shorter hairs of fruit receptacle and spread hairs on the peduncle than *Geum japonicum* Thunb. [30]. In the case of GA, which shows a high total phenol content, studies have demonstrated antioxidant and antiaging effects on extracts and fractions [31].

The total flavonoid content is expressed as quercetin equivalents in milligrams per gram of extracts. Flavonoids are a group of plant secondary metabolites containing hydroxyl groups in their chemical structure. The basic skeleton of flavonoids is composed of two phenyl rings (A and B rings) and one heterocyclic ring (C ring) containing embedded oxygen atoms [32]. These structural features indicate their antioxidant activity; in particular, flavonoid aglycones, such as quercetin, kaempferol, and catechin, have important roles in radical scavenging [33]. Quercetin is one of the most abundant flavonols in plants and has several activities such as antibacterial, anti-inflammatory, neuroprotective, and anticancer effects [34, 35]. In the present study, the range of the total flavonoid content was 7.78–23.61 mg QE/g extract, which was the highest in PD2. Unlike the total phenol content, the total flavonoid content in the cotyledon was higher than that in the true leaves. Kim et al. [36] investigated the total flavonoid content from the bark extract of PD, and the content was 248.7 mg catechin equivalents/g dry weight. In addition, the needles of PD have antimutagenic and antiproliferative effects on cancer cells [37].

Table 4: Total contents of phenol and flavonoid from germinated shoot extracts.

Sample	Phenol (mg TAE/g extract)	Flavonoid (mg QE/g extract)
Cotyledon of *Achyranthes bidentata* (AB1)	19.55 ± 0.67	16.75 ± 1.09
True leaves of *Achyranthes bidentata* (AB2)	21.67 ± 0.78	21.99 ± 1.50
Cotyledon of *Securinega suffruticosa* (SS1)	55.10 ± 1.62	17.37 ± 0.97
True leaves of *Securinega suffruticosa* (SS2)	54.94 ± 0.05	16.89 ± 0.13
Cotyledon of *Chamaecyparis obtusa* (CHA1)	43.45 ± 1.08	19.10 ± 0.38
True leaves of *Chamaecyparis obtusa* (CHA2)	37.87 ± 1.05	18.96 ± 1.09
Cotyledon of *Allium dumebuchum* (AD1)	27.02 ± 0.51	18.14 ± 1.29
True leaves of *Allium dumebuchum* (AD2)	30.47 ± 0.70	10.40 ± 0.96
Cotyledon of *Geum aleppicum* (GA1)	78.88 ± 7.83	19.45 ± 1.29
True leaves of *Geum aleppicum* (GA2)	84.05 ± 2.88	7.78 ± 0.52
Cotyledon of *Cryptotaenia japonica* (CJ1)	15.56 ± 0.83	23.38 ± 1.71
True leaves of *Cryptotaenia japonica* (CJ2)	18.88 ± 0.24	21.68 ± 1.12
Cotyledon of *Ulmus pumila* (UP1)	33.66 ± 6.50	20.55 ± 1.32
True leaves of *Ulmus pumila* (UP2)	49.44 ± 1.22	17.32 ± 2.53
Cotyledon of *Pinus densiflora* (PD1)	15.48 ± 0.96	17.01 ± 1.40
True leaves of *Pinus densiflora* (PD2)	12.75 ± 0.46	23.61 ± 1.52

3.4. Anticancer Effect of Shoot Extracts. One of the characteristics of cancer cells is rapid growth and uncontrollable proliferation [38]. The cytotoxic effect of the germinated shoot extract was confirmed using the AGS human gastric cancer cell line via the MTT assay. PD1 and CHA2 inhibited cancer cell growth by 41.45% and 39.86%, respectively (Table 5). In addition, AB1, AB2, SS2, and CHA1 inhibited cancer cell growth by 28.32%–31.17%; conversely, SS1, AD1, GA1, GA2, CJ1, CJ2, and UP2 did not elicit anticancer effects on AGS cells.

3.5. Quercetin and Vitamin C Contents of Shoot Extracts. Quercetin and vitamin C were quantitatively analyzed by using each elution system through HPLC-UV. The calibration curves were constructed by plotting the peak area against the concentration through linear regression analysis. The calibration curves of quercetin and ascorbic acid showed equations $Y = 32290X - 26879$ and $Y = 21716X - 30438$, respectively. The correlation coefficients (r^2) of quercetin and vitamin C were 0.998 and 0.9991, respectively. The HPLC chromatograms of the extracts and standards are shown in Figures 3 and 4. As a result, the quercetin contents in the germinated shoot extracts were in the range of 0.08–0.23 mg/g extract; in some cases, it was not detected (Table 6). Among the extracts, CHA2 showed the highest content (0.23 mg/g extract), followed by CHA1 (0.18 mg/g extract). The quercetin contents from UP2 and SS2 were also high.

Lee et al. [39] assessed the antioxidant activity and components of CHA leaf extracts that can be applied as functional resources. They conducted HPLC analysis of the aglycone fraction of CHA leaf extracts and found three peaks (quercetin, taxifolin, and kaempferol) in chromatograms. In addition, the tyrosinase inhibitory activity of the aglycone fraction is four times higher than that of arbutin, which is known as a whitening agent. As such, the quercetin content and activities of CHA indicate its applicability as a functional resource.

Table 5: Anticancer effect of germinated shoot extracts.

Sample (100 μg/mL)	Growth inhibition (%)
Cotyledon of *Achyranthes bidentata* (AB1)	28.82 ± 0.25^{e}
True leaves of *Achyranthes bidentata* (AB2)	31.17 ± 0.34^{c}
Cotyledon of *Securinega suffruticosa* (SS1)	ND
True leaves of *Securinega suffruticosa* (SS2)	29.67 ± 0.28^{d}
Cotyledon of *Chamaecyparis obtusa* (CHA1)	28.32 ± 0.26^{e}
True leaves of *Chamaecyparis obtusa* (CHA2)	39.86 ± 0.59^{b}
Cotyledon of *Allium dumebuchum* (AD1)	ND
True leaves of *Allium dumebuchum* (AD2)	11.41 ± 0.42^{f}
Cotyledon of *Geum aleppicum* (GA1)	ND
True leaves of *Geum aleppicum* (GA2)	ND
Cotyledon of *Cryptotaenia japonica* (CJ1)	ND
True leaves of *Cryptotaenia japonica* (CJ2)	ND
Cotyledon of *Ulmus pumila* (UP1)	11.48 ± 0.63^{f}
True leaves of *Ulmus pumila* (UP2)	ND
Cotyledon of *Pinus densiflora* (PD1)	41.45 ± 0.70^{a}
True leaves of *Pinus densiflora* (PD2)	5.77 ± 0.44^{g}

Values are expressed as mean ± SD. $^{a-g}$Means with different letters indicate significant differences ($p < 0.05$) by Duncan's multiple range test. ND, not detected.

Vitamin C analysis (Table 6) revealed that vitamin C content was the highest in the extract of GA1 (1.45 mg/g extract), followed by SS1 (0.86 mg/g extract). The vitamin C contents in the extracts of the cotyledon and the true leaves did not significantly differ. SS has various compounds, including phenolic compounds, flavonoids, alkaloids, and fatty acid, and it elicits antioxidant and antiaging effects [40, 41]. In addition, Park et al. [42] isolated new alkaloids, namely, securinigines A–G, from SS.

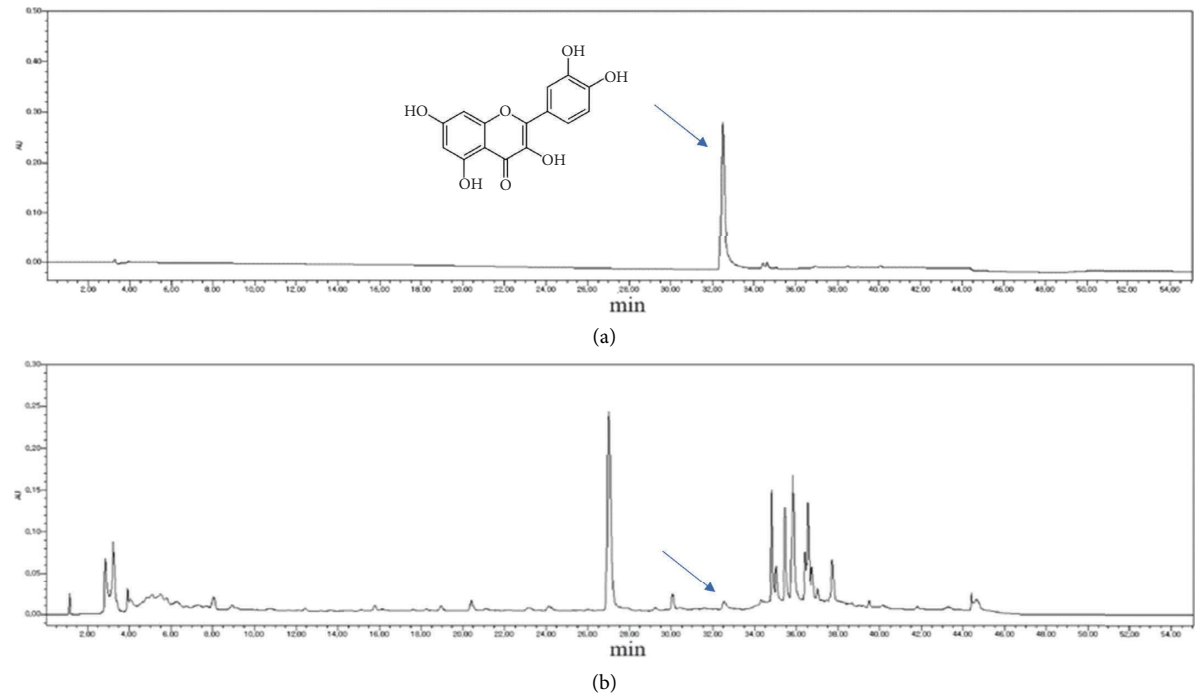

Figure 3: HPLC chromatograms of (a) quercetin and (b) EtOH extract of true leaves of *Chamaecyparis obtusa* (CHA2).

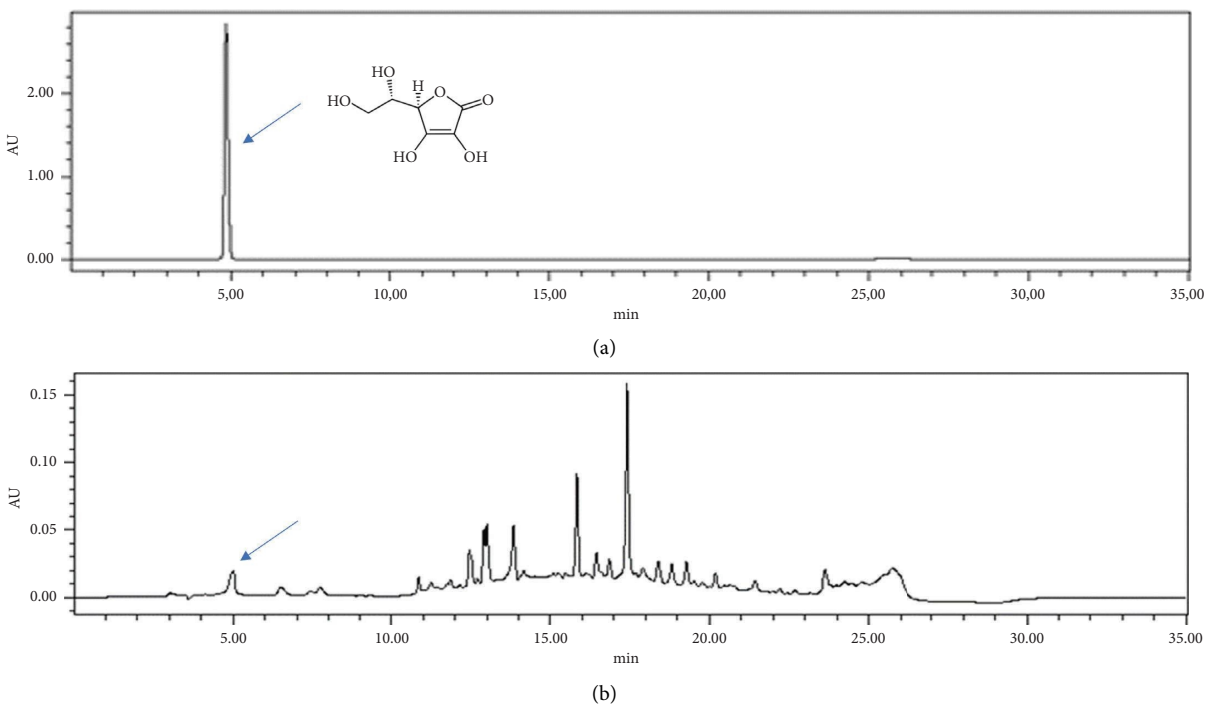

Figure 4: HPLC chromatograms of (a) vitamin C and (b) EtOH extract of cotyledon of *Geum aleppicum* (GA1).

Table 6: Contents of quercetin and vitamin C from germinated shoot extracts.

Sample	Quercetin (mg/g extract)	Vitamin C (mg/g extract)
Cotyledon of *Achyranthes bidentata* (AB1)	0.09 ± 0.02	0.36 ± 0.00
True leaves of *Achyranthes bidentata* (AB2)	0.10 ± 0.01	0.37 ± 0.00
Cotyledon of *Securinega suffruticosa* (SS1)	0.09 ± 0.00	0.86 ± 0.00
True leaves of *Securinega suffruticosa* (SS2)	0.15 ± 0.00	0.40 ± 0.00
Cotyledon of *Chamaecyparis obtusa* (CHA1)	0.18 ± 0.01	0.39 ± 0.00
True leaves of *Chamaecyparis obtusa* (CHA2)	0.23 ± 0.00	0.36 ± 0.00
Cotyledon of *Allium dumebuchum* (AD1)	ND	ND
True leaves of *Allium dumebuchum* (AD2)	ND	0.36 ± 0.00
Cotyledon of *Geum aleppicum* (GA1)	ND	1.45 ± 0.00
True leaves of *Geum aleppicum* (GA2)	ND	0.46 ± 0.00
Cotyledon of *Cryptotaenia japonica* (CJ1)	ND	0.38 ± 0.00
True leaves of *Cryptotaenia japonica* (CJ2)	ND	0.37 ± 0.00
Cotyledon of *Ulmus pumila* (UP1)	0.11 ± 0.01	0.43 ± 0.00
True leaves of *Ulmus pumila* (UP2)	0.17 ± 0.01	0.49 ± 0.00
Cotyledon of *Pinus densiflora* (PD1)	0.08 ± 0.01	0.39 ± 0.00
True leaves of *Pinus densiflora* (PD2)	0.15 ± 0.01	0.39 ± 0.00

Values are expressed as mean ± SD. ND, not detected.

4. Conclusion

This study evaluated the antioxidant, anticancer, and neuroprotective activities of germinated shoots (cotyledon and true leaves) of wild plants and measured their total phenol, flavonoid, quercetin, and vitamin C contents. Our results demonstrated that the GA ethanol extract exerts strong DPPH and ABTS radical scavenging activities. GA treatment also significantly increased cell viability against H_2O_2-induced oxidative stress in SH-SY5Y neuronal cells. In AGS gastric cancer cells, the PD1 and CHA2 significantly inhibited cell proliferation. The total phenol and flavonoid contents were the highest in the shoots of GA and PD2, respectively. The quercetin and vitamin C contents were the highest in CHA2 and GA1, respectively. In conclusion, our findings suggested the antioxidant, anticancer, and neuroprotective activities of the germinated shoots of wild plants and their high total phenol, flavonoid, quercetin, and vitamin C contents. These results were noteworthy in the case of the germinated shoot of GA and provided a basis for studying functional forestry income resources.

Data Availability

The data used to support the findings of this study are available from the corresponding authors upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Juree Kim and Ah Young Lee were responsible for investigation, methodology, and original draft preparation. Chung Ho Choi was responsible for investigation and material preparation. Sanghyun Lee was responsible for conceptualization, project administration, methodology, and review and editing. Juree Kim and Chung Ho Choi contributed equally to this article.

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