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Comparative Analysis of Chemical Composition and Radical-Scavenging Activities in Two Wheat Cultivars

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Abstract: *Triticum aestivum* (wheat) is one of the most significant crops worldwide. This study compares the chemical composition and radical-scavenging activities of two cultivars of *T. aestivum*, Saekeumkang wheat (SW) and Baekkang wheat (BW). Sprouted wheatgrass extracts of SW and BW were analyzed using assessments of total polyphenol and flavonoid contents, liquid chromatography–electrospray ionization/mass spectrometry (LC-ESI/MS), and high-performance liquid chromatography with a photodiode array (HPLC-PDA). Radical-scavenging activities were evaluated using 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{\cdot+}$) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. The results indicated that SW had a higher total polyphenol content than BW, while no significant differences were observed regarding total flavonoid content. HPLC-PDA analysis, guided by LC-ESI/MS, identified four compounds—saponarin, schaftoside, isoorientin, and isovitexin—with isoorientin (3.02 mg/g extract) and schaftoside (4.23 mg/g extract) present in higher concentrations in SW compared to BW. In the $ABTS^{\cdot+}$ assay, the two samples did not show noticeable differences, with SW displaying a scavenging ability with an IC_{50} of 3.36 mg/mL, and BW with an IC_{50} of 3.19 mg/mL. Contrarily, the DPPH assay results showed an inverse trend, suggesting that the radical-scavenging behavior may be influenced by the synergistic and antagonistic interactions of the compounds in SW and BW extracts.

Keywords: *Triticum aestivum*; HPLC/PDA; phytochemical; total phenolic content; antioxidant activity

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1. Introduction

Wheat, rice, corn, and barley are major staple foods globally [1]. More than two-thirds of the world's inhabitants depend on rice as a staple food [2], including being preferred by Korean customers [3]. Wheat, *Triticum aestivum* L. (TAL), is widely cultivated and the second most significant staple food globally [4], and has a long history of farming, starting between 8000 and 10,000 BC [5]. TAL is a member of the family Poaceae and is known for its high content of various nutrients, including vitamins A and C [6]. The Poaceae family, which includes about 11,000 species, displays the unique characteristic of a seed coat merged with an outer layer of the ovary [7]. In the Republic of Korea, the average person consumes about 32 kg of wheat per year, most of which is imported from other countries, such as the United States of America (USA) or Canada [8]. In addition to being a food crop, TAL is an allelopathic crop that can be utilized for weed control [9]. Moreover, wheatgrass has the potential to alleviate osteoporosis, for example, by inhibiting dendritic cell-specific transmembrane protein and cathepsin K [10].

There are several studies regarding the chemical composition of TAL grain and grass. The grain of TAL contains several phenolic compounds. For instance, McKeehen et al. [11]

analyzed the phenolic profiles of six TAL cultivars using high-performance liquid chromatography (HPLC), and found that ferulic and *p*-coumaric acids were primary constituents. In addition, Kowalska et al. [12] isolated 14 phenolic compounds from the aerial parts of TAL, including luteolin derivatives, such as isoorientin (luteolin-6-*C*-glucoside). The young grass of the wheat plant has also been shown to contain various other substances, such as chlorophyll and vitamins [13]. Ghumman et al. [14] conducted a HPLC analysis to identify the chemical composition of TAL grass and found that it contained phenolic compounds, such as gallic acid, catechin, and chlorogenic acid. Furthermore, Luyen et al. [15] isolated eight compounds, including isoorientin, isoscoparin, and daucosterol, from a methanol (MeOH) extract of TAL sprouts. However, there is still a lack of research about the chemical composition and biological functions about the two less-studied cultivars, namely Saekeumkang wheat and Baekkang wheat.

Sprouted TAL, or wheatgrass, is widely utilized as a juice for human consumption owing to its potential pharmaceutical uses, such as managing thalassemia and hemolytic anemia [16]. In addition, wheatgrass juice has become popular globally as a nutritional supplement, as it contains valuable nutrients, for example, amino acids and vitamins [17].

Several studies have demonstrated the nutritional and other benefits of sprouted vegetables, such as buckwheat and barley sprouts. For example, buckwheat sprouts have been studied for their abundance of minerals and amino acids; in addition, they possess higher rutin contents than buckwheat [18]. The phytochemicals that have been isolated from the seeds of tartary buckwheat include kaempferol, quercetin and kaempferol-3-*O*-rutinoside [19]. The results from an oral in vivo assay on inhibited rats confirmed that the flavonoids isolated from buckwheat sprouts conferred anti-stress effects [20]. Due to their flavor and scent, buckwheat sprouts are utilized as a food ingredient, for example, in salads [21]. Likewise, barley grass is a rich source of various components, such as vitamins, proteins, chlorophyll, and saponarin [22]. While comparing the chemical composition of the fermented and unfermented sprouts of barley, Uy et al. [23] have performed a HPLC/PDA analysis to identify compounds such as lutonarin, saponarin and isoorientin. Furthermore, it has shown potential as a nutraceutical, as its juice exhibits antioxidant and anti-obesity activity [24]. In addition, Li et al. [25] found that barley grass juice alleviated tumor development in a hepatocellular carcinoma model. As a result of the germination process, the phenolic contents in grains can be altered [26].

In addition, TAL grass has been studied as a treatment for diseases and a dietary supplement [27]. Previous studies have demonstrated that the shoots of TAL have many health benefits, such as being anti-carcinogenic, anti-inflammatory, inhibiting ulcers, preventing arthritis, acting as an antioxidant, and demonstrating anti-aging activities [28,29]. In a study by Kothari et al. [30], TAL grass juice, which contains flavonoids, triterpenoids, and saponins, was administered to rats with hypercholesterolemia and effectively prevented hyperlipidemia by excreting excess cholesterol.

Wheats take between 6 and 10 days to germinate and become wheatgrass [31]. In addition to wheat sprouts, other plant sprout foods, including broccoli and buckwheat sprouts, have been studied for their biological activities [32]. During germination, many compounds, nutrients, and secondary metabolites, including vitamins, minerals, and phenolic compounds, are produced [31,33]. However, further research is needed to better understand how sprouting contributes to and enhances the nutritional value of foods [33]. Therefore, this study aimed to profile the phytochemicals and bioactive properties of the TAL sprouts using liquid chromatography–electrospray ionization/mass spectrometry (LC-ESI/MS), HPLC, total phenolic/flavonoid content, and antioxidant activities.

2. Materials and Methods

2.1. Plant Materials and Cultivation of Sprouts

The seeds of the TAL cultivars, Saekeumkang wheat (SW) and Baekkang wheat (BW), were obtained from Jeonbuk State Agricultural Research & Extension Services, Iksan, Republic of Korea. SW and BW samples were deposited at Jeonbuk State Agricultural

Research & Extension Services, Iksan, Republic of Korea. The SW and BW seeds were sown in a cultivation box [300 (L) × 220 (W) × 80 (H) mm] and soaked for 12 h, with 3 replicates, on 1 November 2023. They were allowed to germinate in darkness for 2 days after seeding. From the 3rd day, when sprouts began to appear, light-emitting-diode white light was applied for 8 h a day; and hydroponic cultivation was performed with a circulation system of 2 min per hour. The temperature was managed at 20–22 ± 1 °C with 65% relative humidity. The upper part of the seedling that emerged from the seed was uniformly cut using a knife. On the 13th day after sowing, wheatgrass was harvested, dried at 70 °C for 2 days, and then ground. The ground wheatgrass samples have been immediately used for analysis after extraction.

2.2. Instruments and Reagents

Quantitative analyses were conducted using a Waters Alliance e2695 HPLC Separation Module with a Waters 2998 photodiode array (PDA) detector (both Waters Corp., Milford, MA, USA). The J'sphere ODS-H80 column (250 × 4.66 mm, S-4 μm) was purchased from YMC Co., Ltd. (Asan, Republic of Korea), and the trifluoroacetic acid was obtained from Thermo Scientific Inc. (Waltham, MA, USA). The HPLC-grade water and MeOH were obtained from Honeywell (Burdick and Jackson, Muskegon, MI, USA). Folin and Ciocalteu's phenol reagent, aluminum chloride hexahydrate, and sodium carbonate have been purchased from Sigma Aldrich (Saint Louis, MO, USA). 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid (ABTS·$^{+}$) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) have been acquired from Thermo Fisher Scientific Inc. (Waltham, MA, USA). Syringic acid (**1**), saponarin (**2**), 3-feruloylquinic acid (**3**), schaftoside (**4**), isoorientin (**5**), isoschaftoside (**6**), vitexin-2-*O*-rhamnoside (**7**), isovitexin (**8**), scoparin (**9**), and 2-hydroxycinnamic acid (**10**) were supplied by Natural Product Institute of Science and Technology (www.nist.re.kr; accessed on 3 March 2024), Anseong, Republic of Korea (Figure 1).

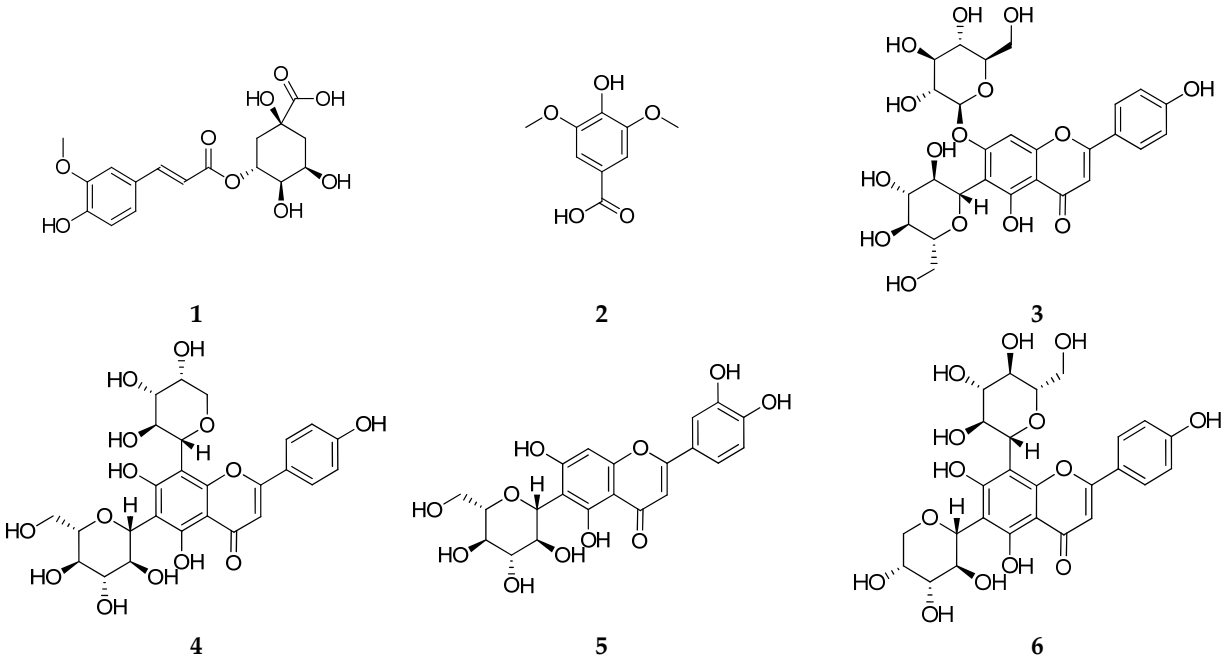


Figure 1. *Cont.*

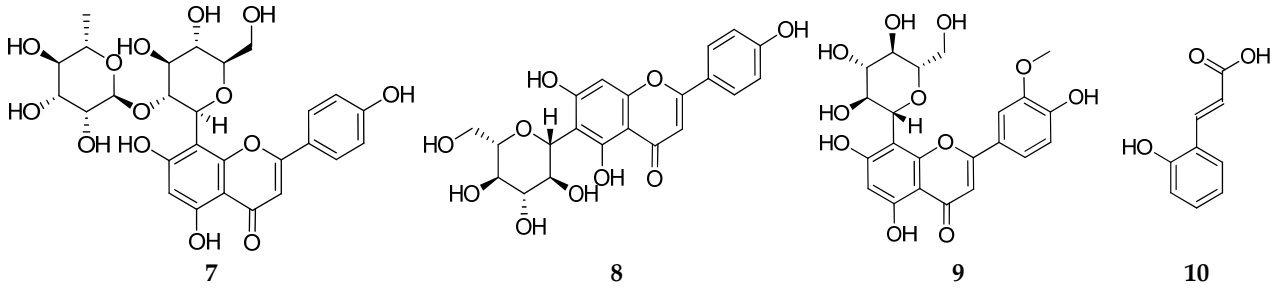


Figure 1. Chemical structures of syringic acid (**1**), saponarin (**2**), 3-feruloylquinic acid (**3**), schaftoside (**4**), isoorientin (**5**), isoschaftoside (**6**), vitexin-2-*O*-rhamnoside (**7**), isovitexin (**8**), scoparin (**9**) and 2-hydroxycinnamic acid (**10**).

2.3. Extraction of TAL Samples

The extraction method of samples was selected based on the evaluation of an appropriate solvent for high antioxidant activity conducted by Kulkarni et al. [34]. Dried samples (10 g) of the sprouted cultivars were extracted using a Soxhlet extractor (Mtops Co., Seoul, Republic of Korea) with 200 mL of ethanol (EtOH). The extraction procedure was repeated thrice. The extracted solvent was concentrated using a rotary evaporator (Eyela, Tokyo, Japan) to obtain a solid TAL wheatgrass extract.

2.4. Total Phenolic Content (TPC)

The solid TAL wheatgrass samples (10 mg) were dissolved in 5 mL of water to a concentration of 2 mg/mL to prepare SW and BW test solutions. Tannic acid was prepared as a standard at a concentration of 1 mg/mL and then diluted to concentrations ranging between 6.25 and 200 ppm to construct a standard calibration curve. First, 60 µL of standard or test solution was applied to a 96-well plate and 40 µL of 2N Folin and Ciocalteu's phenol reagent was added to each well. Next, 100 µL of 7.5% sodium carbonate (Na_2CO_3) solution was added to each well and the plate was gently tapped to mix the solution well. Finally, the solution was mixed using a microplate shaker (FINEPCR Co., Gunpo, Republic of Korea) and measured using a microplate reader (BioTek, Winooski, VT, USA) at 760 nm after being incubated for 30 min at room temperature in the dark. The total phenolic content of samples was calculated as mg tannic acid (TA)/g units.

2.5. Total Flavonoid Content (TFC)

Quercetin was used as the standard for determining the flavonoid content. To create a standard calibration curve, 1 mg of quercetin was dissolved in 80% EtOH to a concentration of 1 mg/mL, and subsequently diluted to solutions between 3.9 and 125 ppm. Five mg of each sample was dissolved in 5 mL of 80% EtOH to a concentration of 1 mg/mL. The standard and test solutions were filtered through a 0.45 µm polyvinylidene fluoride (PVDF) membrane filter before use. Then, 100 µL of standard or test solutions were pipetted into the wells of a 96-well plate, followed by adding 100 µL of 2% aluminum chloride hexahydrate and mixing with a microplate shaker. The reaction was measured using a microplate reader at an absorbance of 430 nm after being incubated for 10 min at room temperature in complete darkness. The total flavonoid content of samples was calculated as mg quercetin (QE)/g units.

2.6. ABTS·$^+$ Radical-Scavenging Activity

The ABTS·$^+$ radical-scavenging assay was conducted according to the protocol of Doan et al. [35]. To assess the ABTS·$^+$ radical-scavenging activity of the TAL extracts, test solutions of the two cultivars were made by dissolving 50 mg of each TAL EtOH extract in water. Then, 10 µL of these solutions were added to a 96-well plate, followed by 200 µL of ABTS·$^+$ working solution. The solutions were mixed using a microplate shaker and then allowed to react in the dark for 30 min. Absorbance was measured at 734 nm using a

microplate reader. Each test was performed in triplicate; the $ABTS^{\cdot+}$ working solution was replaced with water for the blank test. Ascorbic acid served as the standard. The radical-scavenging activity of samples was indicated as a half-maximal inhibitory concentration (IC_{50}) value (mg/mL).

2.7. DPPH Radical-Scavenging Activity

The DPPH radical-scavenging assay was conducted as described by So et al. [36]. First, 50 mg of each TAL EtOH extract was dissolved in EtOH and filtered through a PVDF membrane filter to prepare the test solution. Then, 10 µL of the prepared test solutions were added to a 96-well plate, followed by 200 µL of DPPH working solution. The plate was agitated using a microplate shaker and left to react in the dark for 30 min. Absorbance was measured at 514 nm using a microplate reader. The procedure was repeated three times for each sample, with EtOH replacing the DPPH working solution for the blank test. Ascorbic acid was used as the standard and control group.

2.8. Conditions for LC-ESI/MS

A Vanquish ultra-HPLC system (Thermo Scientific Inc. (Waltham, MA, USA) with a Cortex T3 column (150 × 2.1 mm, 16 µm particle size, Waters Corp., Milford, MA, USA) kept at 45 °C was used for separation. The mobile phase consisted of water with 0.1% formic acid (eluent A) and acetonitrile with 0.1% formic acid (eluent B). The total run time was 55 min: the gradient started at 3% eluent B, ramping up to 15% over 15 min, then climbing to 100% over the next 35 min, and holding for an additional 5 min. After that, the column was re-equilibrated to 3% eluent B for 5 min. A constant flow rate of 0.25 mL was maintained. A heated electrospray ion source equipped with a high-resolution mass spectrometer (HRMS) was used for the analysis. The HRMS collected full-scan MS spectra (m/z 100–1500) with a resolution of 70,000 using a quadrupole system, while operating in positive and negative modes. For the positive ion mode, the spray voltage was adjusted to 3.5 kV, while for the negative ion mode, it was set to 3.0 kV. For the MS2 fragmentation, the 10 most intense precursor ions were chosen, and the spectra were acquired at a resolution of 17,500. Additional MS parameters included a capillary temperature of 320 °C, sheath gas at 50 astronomical units (AU), sweep gas at 1 AU, and auxiliary gas at 10 AU.

2.9. Preparation of Standard and Sample Solutions for HPLC Analysis

The TAL EtOH extracts were prepared to test solutions of 10 mg/mL by dissolving 30 mg of the extracts in 3 mL of MeOH and filtering them through a 0.45 µm PVDF membrane filter before HPLC analysis. Each standard was prepared to 1 mg/mL by dissolving it in MeOH.

2.10. Conditions for HPLC/PDA

The quantitative HPLC/PDA analysis was conducted using a Waters Alliance e2695 HPLC Separation Module and Waters 2998 PDA detector. A J'sphere ODS-H80 column was selected as the stationary phase. The flow rate was maintained at 1 mL/min, the injection volume was set at 10 µL, and the ultraviolet (UV) wavelength was fixed at 254 nm. For the mobile phase, 0.1% of trifluoroacetic acid in water (A) and acetonitrile (B) were used, and the gradient elution system was arranged as follows: 88% A between 0 and 8 min, 80% A at 20 min, 75% A at 40 min, 0% A at 45 to 50 min, and 88% A between 53 and 65 min.

2.11. Calibration Curves

The standard solutions were serially diluted to produce calibration curves. The area of the calibration curve's standard peak was indicated by the Y-axis values (AU), while the X-axis values (µg/mL) represented the standards' concentration. The calculation of the total content of the standards (mg/g) involved multiplying the concentration of the standard (C), the total volume of the test solution (V), the dilution factor (D), and the standard purity (P), then dividing the result by the sample weight (W).

2.12. Statistical Analysis

The experimental results are presented as the mean and standard deviation. Statistical significance was tested using the Minitab 16 software (Minitab LCC, State College, PA, USA), with an analysis of variance (ANOVA), followed by Duncan's multiple range test.

3. Results

3.1. TPC and TFC

TPC and TFC were performed to gather preliminary profile information about the TAL samples before conducting an in-depth chemical composition analysis. The assay results demonstrated that the TPC of SW was higher compared with the BW, at 38.3 and 32.7 mg/g, respectively (Table 1). In contrast, no significant difference was seen in the TFC of the SW and BW cultivars, with 12.5 and 14.8 mg/g, respectively (Table 1). Based on these results, it was anticipated that the cultivars' flavonoid compounds, determined by HPLC/PDA, would be similar.

Table 1. TPC and TFC in the sprouts of SW and BW wheats.

Sample	TPC (mg TAE/g)	TFC (mg QE/g)
SW	38.3 ± 1.7 [a]	12.5 ± 2.3 [a]
BW	32.7 ± 1.4 [b]	14.8 ± 2.0 [a]

TAE, tannic acid equivalent; QE, quercetin equivalent; [a,b] lowercase letters indicate statistically significant differences.

3.2. LC-ESI/MS

The LC-ESI/MS analysis results identified ten compounds (Table 2). Figure 2 shows the total ion chromatograms of SW EtOH extract, and the proposed compounds of the LC-ESI/MS data analysis, including saponarin (**2**), schaftoside (**4**), isoorientin (**5**), and isovitexin (**8**), are shown in Table 3.

Table 2. LC-ESI/MS profiling of the sprouts of the SW in positive and negative ionization modes.

Retention Time (min)	MW $[M - H]^-$	MW $[M + H]^+$	Proposed Compound
11.60	367.1	-	3-Feruloylquinic acid [2]
12.61	197.1	-	Syringic acid [2]
14.91	163.0	-	2-Hydroxycinnamic acid [2]
16.21	563.1	-	Isoschaftoside [2]
17.12	593.2	-	Saponarin [2]
17.49	193.1	-	Ferulic acid [2]
17.90	563.1	565.1	Schaftoside [1,2]
19.88	577.2	579.2	Vitexin-2-*O*-rhamnoside [1,2]
19.89	431.1	433.1	Isovitexin [1,2]
20.59	461.1	463.1	Scoparin [1,2]

[1] positive ion mode; [2] negative ion mode. H, additional proton; M, molecular compound; MW, molecular weight.

Table 3. Calibration curves for saponarin (**2**), schaftoside (**4**), isoorientin (**5**), and isovitexin (**8**).

Compound	t_R [a]	Calibration Equation [b]	*R*-Value [c]
2	16.6	-	-
4	17.4	Y = 10902X + 2665.4	1.0000
5	18.0	Y = 21185X − 676.99	1.0000
8	22.1	-	-

[a] retention time; [b] Y = peak area, X = concentration of the standard (μg/mL); [c] *R*-value = correlation coefficient for five data points in the calibration curve.

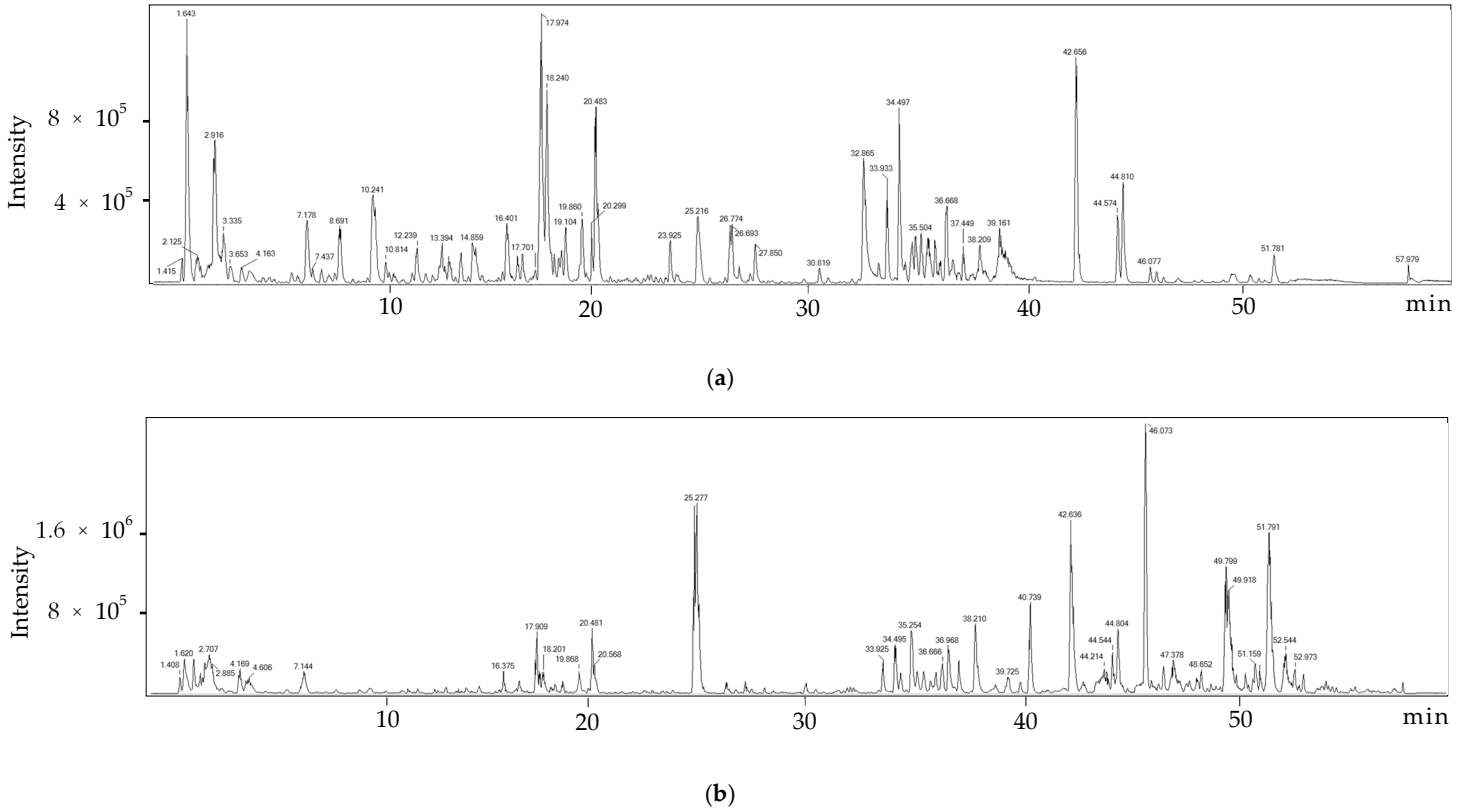


Figure 2. Total ion chromatograms of the sprouts of the SW in negative (**a**) and positive (**b**) ionization modes.

3.3. HPLC/PDA Analysis

Ten compounds were selected for HPLC analysis, based on the LC-ESI/MS data and the literature [37]. Among them, saponarin (**2**), schaftoside (**4**), isoorientin (**5**), and isovitexin (**8**) were detected in the SW and BW extracts. Schaftoside (**4**) and isoorientin (**5**) were quantified according to the calibration curves constructed for each; the calibration curves exhibited good linearity, with a correlation factor of 1 (Table 3). The HPLC/PDA chromatograms of the standards and samples are shown in Figures 3 and 4. The SW exhibited slightly higher contents of schaftoside (**4**) and isoorientin (**5**) compared with BW for the ethanolic extracts and dry-weight samples (Table 4). To illustrate, the schaftoside (**4**) contents of the BW and SW ethanolic extracts were 3.91 and 4.23 mg/g, respectively, and their isoorientin (**5**) contents were 2.53 and 3.02 mg/g, respectively.

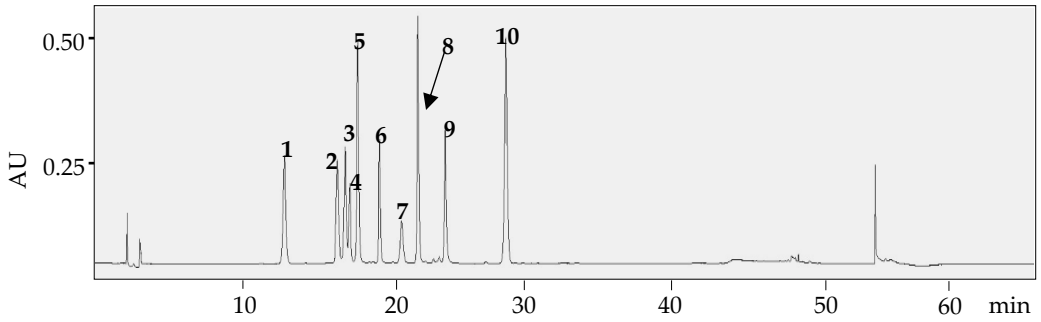
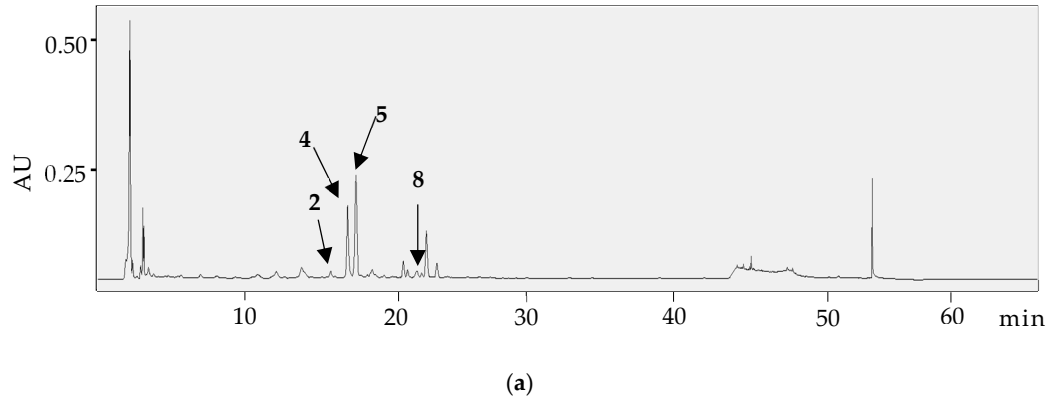
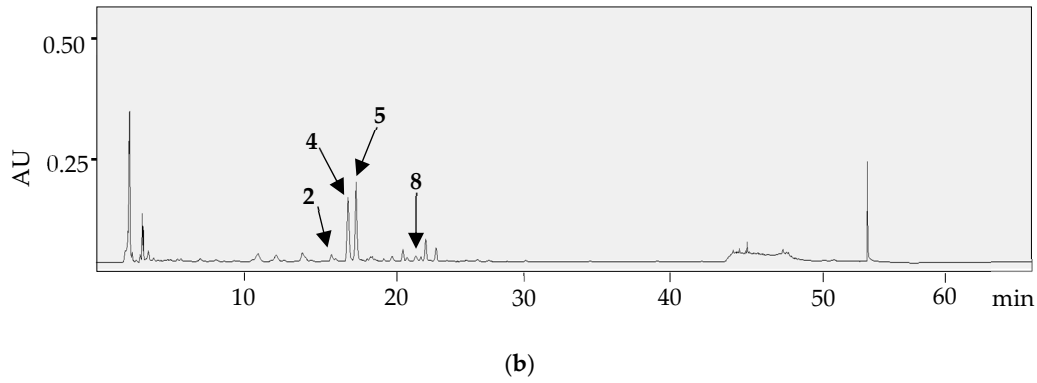


Figure 3. A HPLC/PDA chromatogram of compounds **1**–**10**. The labeled peaks represent syringic acid (**1**), saponarin (**2**), 3-feruloylquinic acid (**3**), schaftoside (**4**), isoorientin (**5**), isoschaftoside (**6**), vitexin-2-*O*-rhamnoside (**7**), isovitexin (**8**), scoparin (**9**), and 2-hydroxycinnamic acid (**10**).



(**a**)



(**b**)

Figure 4. HPLC/PDA chromatograms of the sprouts of SW (**a**) and BW (**b**). The labeled peaks represent saponarin (**2**), schaftoside (**4**), isoorientin (**5**), and isovitexin (**8**).

Table 4. The contents of saponarin (**2**), schaftoside (**4**), isoorientin (**5**), and isovitexin (**8**) in the sprouts of SW and BW.

Compound	SW		BW	
	mg/g Extract	mg/g DW	mg/g Extract	mg/g DW
2	tr	tr	tr	tr
4	4.23 ± 0.05 [a]	1.10 ± 0.01 [a]	3.91 ± 0.03 [b]	0.98 ± 0.01 [b]
5	3.02 ± 0.02 [a]	0.78 ± 0.01 [a]	2.53 ± 0.02 [b]	0.63 ± 0.01 [b]
8	tr	tr	tr	tr

tr, trace amount; [a,b] lowercase letters indicate statistically significant differences.

3.4. Antioxidant Activities

The antioxidant assay results show that BW exhibited slightly stronger antioxidant $ABTS^{\cdot+}$ and DPPH radical-scavenging activities, having half-maximal inhibitory concentration (IC_{50}) values of 3.19 and 14.14 mg/mL, respectively, compared with SW's IC_{50} values of 3.36 and 16.12 mg/mL, respectively (Table 5). BW exhibited slightly stronger antioxidant activities regarding DPPH than SW; however, it did not show significant difference regarding $ABTS^{\cdot+}$.

Table 5. The radical-scavenging activity of the sprouts of SW and BW.

Sample	$ABTS^{\cdot+}$ Radical-Scavenging Activity (IC_{50}, mg/mL)	DPPH Radical-Scavenging Activity (IC_{50}, mg/mL)
SW	3.36 ± 0.09 [a]	16.12 ± 0.40 [a]
BW	3.19 ± 0.33 [a]	14.14 ± 0.51 [b]
AA	0.12 ± 0.00 [b]	0.12 ± 0.00 [c]

AA, ascorbic acid; IC_{50}, half-maximal inhibitory concentration; [a,b,c] lowercase letters indicate statistically significant differences.

4. Discussion

The current study aimed to determine the chemical composition and identify the bioactive activities of two cultivars of TAL to identify potential pharmaceutical and nutraceutical uses. As a result of the HPLC analysis, we also detected compounds in the samples that have been previously studied in other cultivars of wheatgrass [11–15]. Saponarin (**2**), a C-glycosidic flavonoid from *Passiflora incarnata*, is known to lower blood pressure, reduce inflammation, and support cardiac health [22,38]. Isoorientin (**5**), from *Passiflora edulis* leaves, exhibits anti-inflammatory, antinociceptive, antioxidant, antidepressant, and anti-cancer properties, and helps increase bone mineral density in rats [39–43]. Schaftoside (**4**) aids wound healing, has strong antioxidant activity, and regulates melanin production [44–46]. Isovitexin (**8**) is effective in inhibiting α-glucosidase [47].

BW has shown higher DPPH radical-scavenging activity compared to SW, but the two samples were not statistically different regarding the ability to scavenge $ABTS^{\cdot+}$. Also, contrary to expectations that higher total polyphenol contents would also influence the bioactive functions of the two samples, the results of DPPH radical-scavenging activity exhibited an opposite trend. The correlation between various phytochemicals and their antioxidant activities in the wheatgrass samples can be explained by the synergistic and antagonistic effects of antioxidant compounds [48,49]. According to the results of the ferric-reducing antioxidant power assay for compounds, including caffeic acid and quercetin, some combinations produced the most synergistic effects; in contrast, the combination of rutin, rosmarinic acid, and caffeic acid produced antagonistic results (−21.8%) [50]. Moreover, Mao et al. [51] proposed that acteoside found in the flowers of *Osmanthus fragrans* and gallic acid in green tea produced meaningful synergistic effects, which exceeded the sum of their individual activities [52]. The current study has uncovered the chemical information and biological functions of two less-studied cultivars of wheatgrass. It is expected that this research could be a starting point about future potential to industries,

such as pharmaceutical aspects. However, further studies about several aspects are required. For instance, to understand the correlation between the bioactive compounds found in TAL, and its antioxidant activities, further research regarding the synergistic and antagonistic effects of its compounds is needed. Also, to fill diverse information in the gap about these cultivars, studies regarding additional bioactive functions of these cultivars are also needed.

5. Conclusions

This study aimed to determine the chemical composition and bioactive properties of wheatgrass extracts from two TAL cultivars, SW and BW. Analyses using LC-ESI/MS, HPLC/PDA, TPC, and TFC were conducted to profile the SW and BW extracts' chemical composition, and $ABTS^{\cdot+}$ and DPPH radical-scavenging assays were performed to investigate their biological activities. The results revealed that SW exhibited a higher TPC compared with BW but a lower TFC. Four flavonoid compounds, including schaftoside (**4**) and isoorientin (**5**), were detected by HPLC/PDA analysis. The SW displayed stronger $ABTS^{\cdot+}$ radical-scavenging activity (IC_{50}, 3.25 mg/mL) compared with the BW (IC_{50}, 4.00 mg/mL); however, the opposite trend was observed for DPPH radical-scavenging activity. This trend may be explained by the synergistic and antagonistic effects of different combinations of antioxidant compounds. Therefore, future studies are needed to scrutinize the combined effects of the marker compounds for TAL.

Author Contributions: HPLC/PDA, TPC, TFC analysis and radical-scavenging activity, N.Y.; sampling and resources, S.-H.J. and J.-S.P.; LC-ESI/MS analysis, W.J.K.; experimental design and conception, J.-S.P.; supervision, writing—review and editing, S.L. All authors have read and agreed to the published version of the manuscript.

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