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Assessment of Bioactive Compounds and Antioxidant Activity of Barley Sprouts

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Abstract: Barley sprouts, rich in bioactive compounds, have gained attention as functional food ingredients because of their antioxidant potential. This study evaluated their bioactive composition and antioxidant capacity, focusing on the saponarin, chlorophyll, policosanol, total polyphenol (TP), and total flavonoid (TF) contents. The antioxidant capacity was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and ferric-reducing antioxidant power (FRAP) assays. The results showed that barley sprouts contained 8.14 ± 0.02 mg/g of saponarin, 15.36 ± 0.18 mg/g of total chlorophyll, 396.99 mg/100 g of policosanols, 12.64 ± 0.04 mg of gallic acid equivalent (GAE)/g, and 5.99 ± 0.09 mg of rutin equivalent (RE)/g. The half-maximal inhibitory concentration (IC_{50}) values exhibited the trend FRAP > DPPH > ABTS. Significant correlations ($R > 0.891$, $p < 0.05$) were observed between the antioxidant assays and TP and TF contents, indicating their substantial role in the antioxidant properties of barley sprout extracts. These findings suggest that barley sprouts are a valuable natural source of antioxidants for functional food applications. Nevertheless, additional in vivo and clinical research is necessary to improve their bioavailability and expand their potential use in food formulations.

Keywords: barley sprout; saponarin; HPLC-PDA; chlorophylls; policosanols; GC-MS; antioxidants

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1. Introduction

Sprouted grains are consumed globally, and have gained significant attention because they improve the nutritional content of food when used as ingredients [1]. In sprouted grains, various proportions of carbohydrates, proteins, and lipids undergo degradation, converting into simpler and more bioavailable compounds, such as sugars, free amino acids, and fatty acids, respectively [2–5]. Additionally, the germination process can influence the levels of essential minerals, vitamins, and phytochemical compounds, such as polyphenols, phytic acid, enzyme inhibitors, and glucosinolates [6–10].

The increasing demand for health-promoting foods is primarily driven by consumers' interest in improving their well-being, further supported by the growing market for functional ingredient-based products [11]. Researchers are actively exploring new functional ingredients to expand the potential for developing innovative foods with nutritional advantages. Among these, barley powder, which is rich in bioactive compounds and easily incorporable into various food matrices, is considered a promising candidate for this purpose [12]. Sprouting is an inexpensive and sustainable process that enhances the content of functional compounds, digestibility, and bioavailability of grains [13,14].

Barley sprout is recognized as one of the oldest cultivated cereal grains in the world [15]. It is widely cultivated, and ranks as the fourth most produced grain globally, with an annual production of 157 million tons [16]. Barley sprout continues to be a staple grain in certain regions of Asia and North Africa. In recent years, its application as a food ingredient has increased, due to its high nutritional profile and abundance of bioactive compounds [17]. The major phytochemicals found in barley sprouts include phenolic acids, flavonoids, lignans, vitamin E (tocols), sterols, and folates [18]. Previous research has shown that barley sprouts exhibit significant free radical-scavenging activities, which may be attributed to their high levels of polyphenols, flavonoids, and other bioactive compounds [12,19].

This research aimed to analyze the bioactive compounds in barley sprouts by analyzing their saponarin, chlorophyll, and policosanol contents. Additionally, their antioxidant capacity was evaluated using a 2,2-diphenyl-1-picrylhydrazyl (DPPH)-scavenging activity assay, a 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)-scavenging activity assay, and a ferric-reducing antioxidant power (FRAP) assay, along with determining the total polyphenol (TP) and total flavonoid (TF) contents.

2. Materials and Methods

2.1. Barley Sprout Sample and Chemicals

Barley sprouts (*Hordeum vulgare* L.) were freeze-dried at the Agricultural Cooperative University (Goyang, Republic of Korea). The barley sprouts were ground using a blender (SFM-555SP; Shinil Industrial Co., Ltd., Seoul, Republic of Korea) and sieved through a 40-mesh screen before use.

Barley sprouts (500 mg) were precisely measured (500 mg) and transferred into a 100 mL volumetric flask. Next, 100% methanol was introduced to adjust the volume to 100 mL, followed by extraction using a sonicator (JAC-4020, KODO Technical Research Co., Ltd., Hwaseong, Republic of Korea) for 30 min. Following the extraction process, the solution was subjected to centrifugation at 1520× *g* for 30 min to separate the supernatant. The resulting solution was then passed through a 0.45 μm syringe filter to ensure purity before analysis.

To extract policosanols, 1 g of barley sprouts was subjected to shaking extraction in hexane at 25 °C for 24 h. After the extraction, the solution underwent filtration, followed by vacuum evaporation to concentrate the crude extract. For gas chromatography–mass spectrometry (GC-MS) analysis, the extract was derivatized using N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA, Sigma-Aldrich, St. Louis, MO, USA), employing the same protocol as the reference standards.

A precise amount of 10 mg of saponarin (PHL89784; Sigma-Aldrich, St. Louis, MO, USA) was weighed and dissolved in 10 mL of absolute methanol to create a stock solution at a concentration of 1 mg/mL. This solution was stored at 4 °C and further diluted with methanol to obtain calibration standard solutions at concentrations of 6.25, 12.5, 25, 50, and 100 μg/mL.

For identification, the policosanols were derivatized into trimethylsilyl forms. To achieve this, 250 µL of MSTFA was introduced into 0.5 mL of chloroform containing the policosanol standards. The resulting mixture was then agitated at 50 °C for 15 min, after which additional chloroform was added to bring the final volume to 1 mL for subsequent analysis.

To measure the TP and TF contents and antioxidant activity, 1 g of the sample was extracted in 30 mL of methanol:water (1:1, v/v) using a sonicator for 1 h. The extract was then centrifuged at 1520× g for 30 min, filtered through a 0.45 µm syringe filter, and subsequently used for the experiments.

2.2. Analysis of Saponarin

The quantification of saponarin was carried out using high-performance liquid chromatography (HPLC) coupled with a photodiode detection array (PDA), following the protocol outlined by Kim et al. [20]. An HPLC analysis was performed utilizing a Shimadzu LC system (LC-40B XR, Shimadzu Co., Ltd., Kyoto, Japan) integrated with an PDA detector (SPD-M40, Shimadzu Co., Ltd., Kyoto, Japan). Chromatographic separation was achieved using a CAPCELL PAK C18 UG120 column (4.6 mm × 250 mm, 5 µm). The mobile phase consisted of 0.1% triple-distilled water containing 1% formic acid (solvent A) and acetonitrile with 1% formic acid (solvent B). The system operated at a flow rate of 1.0 mL/min, with the column temperature maintained at 40 °C. Sample injections were performed with a volume of 5 µL, and detection was conducted at a wavelength of 254 nm. The specific experimental parameters are summarized in Table 1.

Table 1. HPLC-PDA conditions for saponarin analysis.

Parameters	**Conditions**		
HPLC system	Shimadzu LC system LC-40B XR		
Detection system	Shimadzu SPD-M40 PDA		
Column	CAPCELL PAK C18 UG120 (4.6 mm × 250 mm, 5 µm)		
Column temperature	40 °C		
Flow rate	1.0 mL/min		
Wavelength	254 nm		
Injection volume	5 µL		
Mobile phase	A: DDW containing 1% formic acid		
	B: Acetonitrile containing 1% formic acid		
Gradient	Time (min)	A (%)	B (%)
	0	100	0
	20	90	10
	32	60	40
	36	40	60
	42	90	10

2.3. Analysis of Chlorophylls

Barley sprouts (50 mg) were mixed with 10 mL of a solvent consisting of acetone and methanol (8:2, v/v). The solution underwent vortex mixing, followed by extraction for 1 h at 25 °C under dark conditions. After the extraction step, 1.5 mL of the resulting extract was subjected to centrifugation at 15,680× g for 10 min, and the supernatant was carefully separated. The remaining pellet was resuspended in 1 mL of the same acetone–methanol solvent (8:2, v/v), vortexed thoroughly, and re-centrifuged at 15,680× g for 10 min. The final supernatant was collected and pooled together with the previously obtained extracts. The combined extract was analyzed using a quartz cuvette to measure the absorbance at 645 and 663 nm, using an acetone–methanol solvent (8:2, v/v) as a

blank. The chlorophyll content was calculated based on the measured absorbance values using Equations (1)–(3):

$$\text{Chlorophyll a (mg/g)} = (12.21 \times A_{663\,nm} - 2.81 \times A_{645\,nm}) \times N \times V/(1000 \times M) \quad (1)$$

$$\text{Chlorophyll b (mg/g)} = (20.13 \times A_{645\,nm} - 5.03 \times A_{663\,nm}) \times N \times V/(1000 \times M) \quad (2)$$

$$\text{Total chlorophyll (mg/g)} = \text{chlorophyll a} + \text{chlorophyll b} \quad (3)$$

2.4. Analysis of Policosanols

Policosanols were quantified following the analytical protocol established by Seo et al. [21]. The trimethylsilyl alcohol derivatives were characterized via gas chromatography (GC) coupled with mass spectrometry (MS). An Agilent Technologies 7890A series GC system, integrated with a single quadrupole MS detector (Agilent Technologies, Palo Alto, CA, USA), was utilized for the analysis. Chromatographic separation was performed using an HP-5MS capillary GC column (30 m × 0.25 mm × 0.25 µm film thickness; Agilent Technologies, Santa Clara, CA, USA). The oven temperature was programmed to gradually increase from 150 °C to 325 °C at a controlled rate of 4 °C/min, followed by a 5 min hold at 320 °C. Helium was employed as the carrier gas, flowing at a rate of 1.8 mL/min. Sample injection was conducted with a volume of 1 µL using an autosampler (Agilent Technologies, Santa Clara, CA, USA) and a split ratio of 1:5. For MS detection, the electron impact ion source and transfer line temperatures were set to 200 °C and 280 °C, respectively, with an ionization energy of 70 eV. The detailed experimental conditions are summarized in Table 2.

Table 2. GC-MS conditions for analysis of policosanols.

Parameters	Conditions	
GC system	7890A series GC system coupled with 5975C single quadrupole MS	
Column	HP-5MS capillary column (30 m × 0.25 µm × 0.25 µm film thickness)	
Carrier gas	Helium, 1.8 mL/min	
Temperature program	150 °C to 325 °C with a 4 °C/min heating rate, and then maintained at 320 °C for 5 min	
Inlet	Temperature	280 °C
	Injection volume	1 µL
	Split ratio	1:5
Mass spectra	Ionization mode	Electron impact
	Ionization energy	70 Ev
	Ion source and transfer line temperatures	200 and 280 °C
	Mass range	40–500 amu
	Analytical mode	SCAN and SIM

2.5. Total Polyphenol and Flavonoid Content

The TP content was assessed following the procedure outlined by Duval and Shetty [22]. A reaction mixture was prepared by combining 1 mL of the sample, 2% Folin–Ciocalteu phenol reagent, and 10% Na_2CO_3 in a 1:1:1 ratio, followed by incubation for 1 h. Upon completion of the reaction, absorbance was measured at 750 nm using a microplate reader (Spectramax i3; Molecular Devices Co., Sunnyvale, CA, USA). The TP content was quantified using a gallic acid calibration curve, and expressed as gallic acid equivalent (GAE)/g.

To evaluate the TF content, 0.5 mL of the sample was combined with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum nitrate, 0.1 mL of 1 M potassium acetate, and

2.8 mL of distilled water. The solution was incubated at 25 °C for 30 min, and the absorbance was measured at 415 nm using a microplate reader (Spectramax i3, Molecular Devices Co., Sunnyvale, CA, USA). The TF content was determined using a rutin calibration curve, and expressed as rutin equivalent (RE)/g.

2.6. DPPH Radical-Scavenging Activity

The DPPH radical-scavenging activity assay operates on the principle that the stable free radical DPPH undergoes reduction in the presence of sulfur-containing amino acids, such as cysteine and glutathione, as well as ascorbic acid and aromatic amines. This reduction reaction results in a shift in color from purple DPPH to colorless diphenyl picrylhydrazine, which is accompanied by a corresponding change in absorbance. This method serves as a reliable approach for evaluating the antioxidant capacity of bioactive compounds [23].

A reaction mixture was prepared by combining 200 µL of the sample with 800 µL of 0.4 mM DPPH reagent, and incubating it in darkness for 10 min. The absorbance was recorded at 517 nm using a microplate reader (Spectramax i3, Molecular Devices Co., Sunnyvale, CA, USA). The DPPH radical-scavenging activity was determined based on Equation (4), with the results expressed as the half-maximal inhibitory concentration (IC_{50}).

$$\text{DPPH radical-scavenging activity (\%)} = (\text{Blank O.D} - \text{Sample O.D})/\text{Blank O.D} \times 100 \quad (4)$$

2.7. ABTS Radical-Scavenging Activity

The ABTS radical-scavenging activity assay is a commonly employed method for evaluating antioxidant capacity. This assay is based on the principle that ABTS reacts with potassium persulfate under dark conditions, leading to the formation of ABTS radicals ($ABTS^+$). These radicals are subsequently neutralized by the antioxidant components present in the sample, resulting in the decolorization of the characteristic teal hue associated with $ABTS^+$ radicals [24].

A 7 mM ABTS solution and a 2.45 mM potassium persulfate solution were mixed in a 2:1 ratio, and incubated in the dark for 16 h to facilitate radical generation. The resulting solution was subsequently diluted with anhydrous ethanol in a 1:88 ratio. For the assay, 10 µL of the sample was added to 1 mL of the diluted ABTS solution, and incubated in darkness for 6 min. Following the reaction, absorbance was recorded at 734 nm using a microplate reader (Spectramax i3, Molecular Devices Co., Sunnyvale, CA, USA). ABTS radical-scavenging activity was determined using Equation (5), and the results were expressed as IC_{50}.

$$\text{ABTS radical-scavenging activity (\%)} = (\text{Blank O.D} - \text{Sample O.D})/\text{Blank O.D} \times 100 \quad (5)$$

2.8. FRAP Assay

The FRAP assay operates on the principle that ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex undergoes reduction to Fe^{2+}-TPTZ by a reducing agent under acidic conditions. This method is founded on the concept that most antioxidants exhibit reducing power [25].

To prepare the FRAP reagent, 300 mM acetate buffer (pH 3.6), 10 mM TPTZ, and 20 mM $FeCl_3 \cdot 6H_2O$ were separately prepared and combined in a 10:1:1 ratio. A reaction mixture was then formulated by mixing 1.5 mL of the FRAP solution, 50 µL of the sample, and 150 µL of distilled water. The mixture was incubated at 37 °C for 4 min, after which the absorbance was recorded at 593 nm using a microplate reader (Spectramax i3, Molecular Devices Co., Sunnyvale, CA, USA), and the results were expressed as IC_{50}.

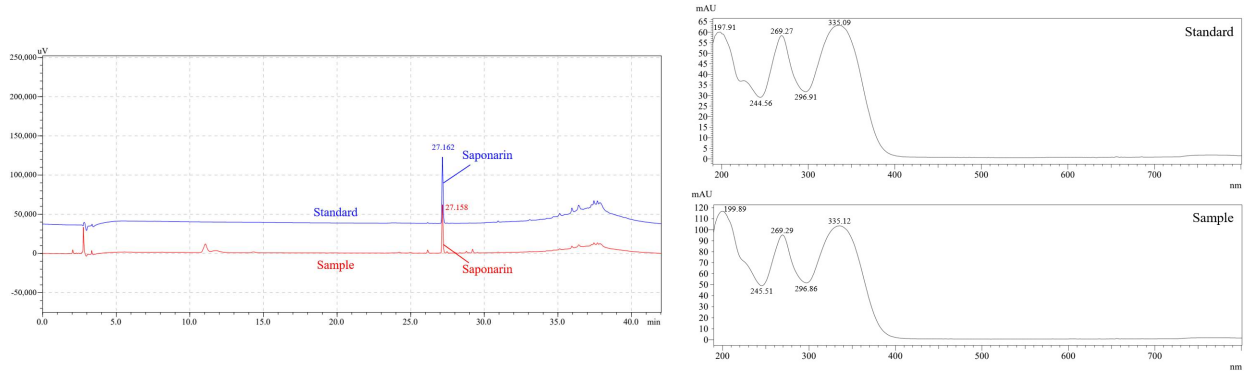
2.9. Statistical Analyses

The experimental data are expressed as means ± standard deviations (SD). Statistical analysis was performed using SPSS software (version 24.0; SPSS Inc., Chicago, IL, USA). The significance of differences among experimental groups was assessed using Duncan's multiple range test, with *p*-values less than 0.05 considered statistically significant. The correlation strength was evaluated using Pearson's correlation coefficient.

3. Results

3.1. Saponarin and Clorophyll Contents in Barley Sprout

The saponarin standard solution was diluted with distilled water to final concentrations of 6.25, 12.5, 25, 50, and 100 μg/mL, establishing a linear concentration range. Standard solutions prepared at each concentration were analyzed using HPLC-PDA, and a calibration curve was constructed to represent the relationship between the concentration and peak area (Figure 1a). The correlation coefficient (R^2) of the calibration curve (y = 7847.7x + 2180.5) exhibited a high linearity, exceeding 0.9999. Furthermore, similarity was confirmed when the UV spectrum of the selected peak from the 25 μg/mL saponarin standard solution and the 5000 μg/mL barley sprout sample was examined (Figure 1b). In this study, we demonstrated the potential of saponarin in barley sprouts as a marker compound, and confirmed the reproducibility of quantitative analysis using a widely applicable HPLC-PDA system.



(**a**) (**b**)

Figure 1. HPLC chromatogram of saponarin standard and barley sprout for saponarin content. HPLC chromatogram of 25 μg/mL saponarin standard and 5000 μg/mL barley sprout sample (**a**). Blue curve is saponarin standard chromatogram and red curve is sample chromatogram. (**b**) UV spectrum of saponarin standard and barley sprout.

The absorbance readings of the centrifuged solution measured at 663 nm and 645 nm indicated that the freeze-dried barley sprout contained 13.10 ± 0.29 mg/g of chlorophyll a, 2.25 ± 0.09 mg/g of chlorophyll b, and 15.36 ± 0.18 mg/g of total chlorophyll, respectively (Table 3).

Table 3. Contents of saponarin and chlorophylls in barley sprout.

	Barley Sprout (mg/g)
Saponarin	8.14 ± 0.02 (RSD [1] 0.25%)
Chlorophyll a	13.10 ± 0.29 (RSD 2.21%)
Chlorophyll b	2.25 ± 0.09 (RSD 4.00%)
Total chlorophyll	15.36 ± 0.18 (RSD 1.17%)

Results are presented as mean ± SD of 3 independent experiments in triplicate. [1] Relative standard deviation.

3.2. Identification and Quantification of Policosanols

Figure 2 presents the GC-MS chromatograms of the policosanol standard solutions alongside those of the barley sprout samples. The retention times of the components in the simultaneous analysis of the standard solution were as follows: eicosanol, 19.481; heneicosanol, 21.373; docosanol, 23.236; tricosanol, 25.015; tetracosanol, 26.766; hexacosanol, 30.069; heptacosanol, 31.650; octacosanol, 33.175; and triacontanol, 36.111.

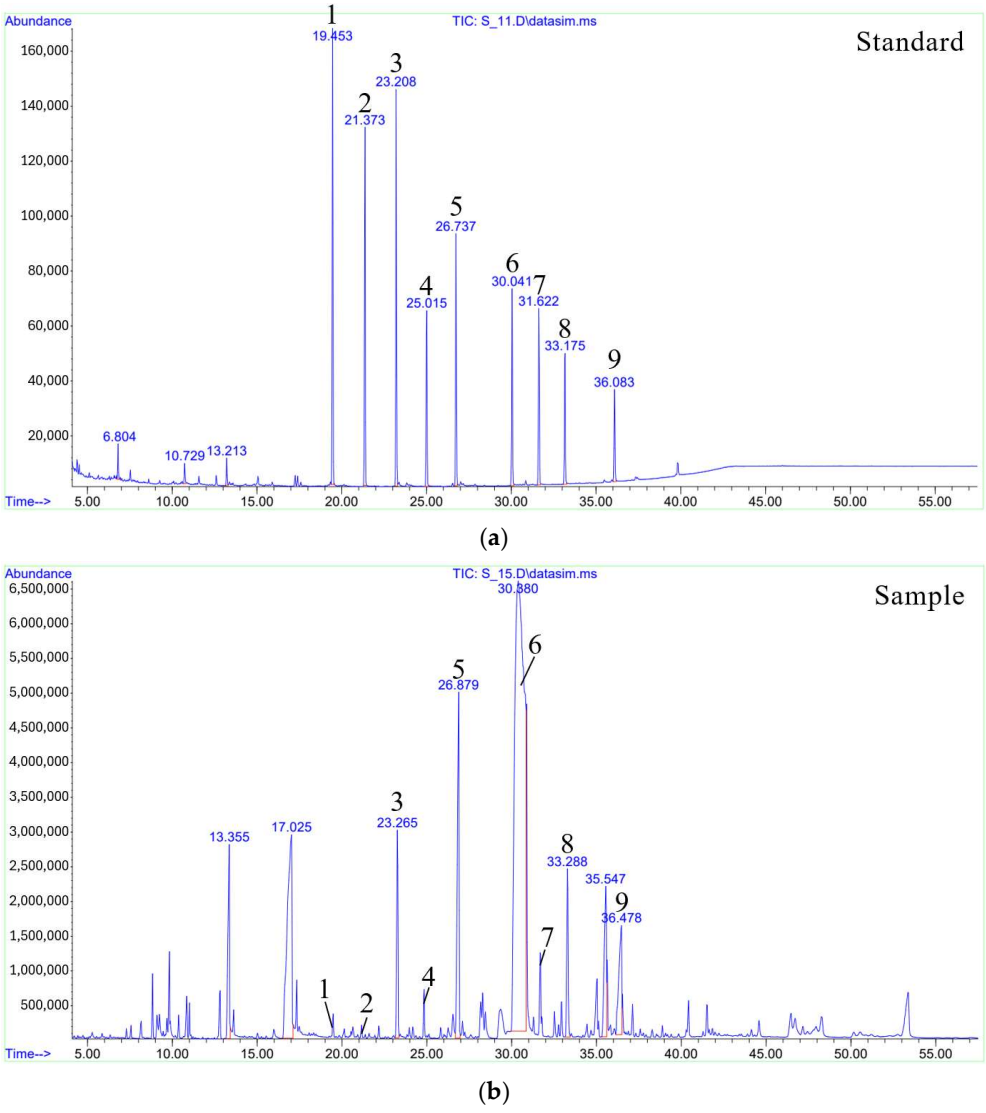


Figure 2. GC-MS chromatogram regarding TMS derivatives of (**a**) policosanol standards and (**b**) barley sprout sample. Peaks: 1, eicosanol; 2, heneicosanol; 3, docosanol; 4, tricosanol; 5, tetracosanol; 6, hexacosanol; 7, heptacosanol; 8, octacosanol; and 9, triacosanol.

The types and contents of the TMS derivatives of policosanols in the silylated barley sprout samples are shown in Table 4. The measured contents were as follows: eicosanol, 1.32 mg/100 g; heneicosanol, 0.45 mg/100 g; docosanol, 15.28 mg/100 g; tricosanol, 3.46 mg/100 g; tetracosanol, 46.65 mg/100 g; hexacosanol, 299.39 mg/100 g; heptacosanol, 8.35 mg/100 g; octacosanol, 19.82 mg/100 g; and triacontanol, 2.27 mg/100 g.

Table 4. Content of policosanol compounds in barley sprout.

Compound	Peak Number	Retention Time	Policosanol—TMS Derivative Mass (*m*/*z*)	Contents (mg/100 g)	Ratio (%)
Eicosanol	1	19.475	355.30 $[M-15]^+$	1.32	0.33
Heneicosanol	2	21.395	369.30 $[M-15]^+$	0.45	0.11
Docosanol	3	23.287	383.40 $[M-15]^+$	15.28	3.84
Tricosanol	4	24.840	397.40 $[M-15]^+$	3.46	0.87
Tetracosanol	5	26.901	411.40 $[M-15]^+$	46.65	11.75
Hexacosanol	6	30.289	439.40 $[M-15]^+$	299.39	75.41
Heptacosanol	7	31.701	453.50 $[M-15]^+$	8.35	2.10
Octacosanol	8	33.310	467.50 $[M-15]^+$	19.82	4.99
Triacontanol	9	36.218	495.50 $[M-15]^+$	2.27	0.57
Total				396.99	

3.3. TP and TF Contents

Polyphenolic compounds encompass various subclasses, including flavonoids, anthocyanins, tannins, catechins, isoflavones, lignans, and resveratrol, which are extensively distributed across the plant kingdom, and predominantly found in fruits and leafy vegetables [26]. Polyphenols and flavonoids possess multiple hydroxyl (–OH) groups, enabling them to interact with reactive oxygen species (ROS) and exhibit antioxidant effects [27]. The results of TP and TF content analysis are summarized in Table 5. The TP content in barley sprout was 12.64 ± 0.04 mg GAE/g, while the TF content was 5.99 ± 0.09 mg RE/g.

Table 5. Phenolic contents in samples.

Sample	Total Polyphenol Content (mg GAE [1]/g)	Total Flavonoid Content (mg RE [2]/g)
Barley sprouts	12.64 ± 0.04	5.99 ± 0.09

Results are presented as mean ± SD of 3 independent experiments in triplicate. [1] GAE: gallic acid equivalent. [2] RE: rutin equivalent.

3.4. Antioxidant Effects of Barley Sprout

The results of the DPPH and ABTS free radical-scavenging activity assays, as well as the FRAP assay, are presented in Table 6. The IC_{50} values for DPPH, ABTS, and FRAP in barley sprout were 21.04 ± 0.00 mg/mL, 88.53 ± 2.75 mg/mL, and 2.50 ± 0.00 μg/mL, respectively, following the order of FRAP > DPPH > ABTS in terms of antioxidant activity. For ascorbic acid, the IC_{50} values for DPPH, ABTS, and FRAP were 0.03 ± 0.00 μg/mL, 0.44 ± 0.01 μg/mL, and 0.01 ± 0.00 μg/mL, respectively. Although ascorbic acid exhibited significantly higher antioxidant activity than barley sprouts across all assays, the trend in activity remained consistent, following the order FRAP > DPPH > ABTS.

Table 6. Antioxidant activities of barley sprout.

Compound	DPPH (IC_{50}, mg/mL)	ABTS (IC_{50}, mg/mL)	FRAP (IC_{50}, mg/mL)
Barley sprout	21.04 ± 0.00	88.53 ± 2.75	2.50 ± 0.00
Ascorbic acid	0.03 ± 0.00	0.44 ± 0.01	0.01 ± 0.00

Results are presented as the mean ± SD of 3 independent experiments in triplicate.

3.5. Relationship Between Antioxidant Activities and Phenolic Contents

Correlation analysis of the total antioxidant capacity values was conducted to evaluate the suitability and reliability of the analytical methods used to measure the antioxidant activity and phenolic content. As shown in Figure 3, all R-values exhibited a positive correlation at a significance level of $p < 0.05$, indicating a strong correlation among antioxidant capacity values. Among them, the correlation between TF and FRAP was the

strongest (R = 0.999), followed by the correlation between ABTS and FRAP, as well as between TF and ABTS, which also exhibited strong relationships (R = 0.998).

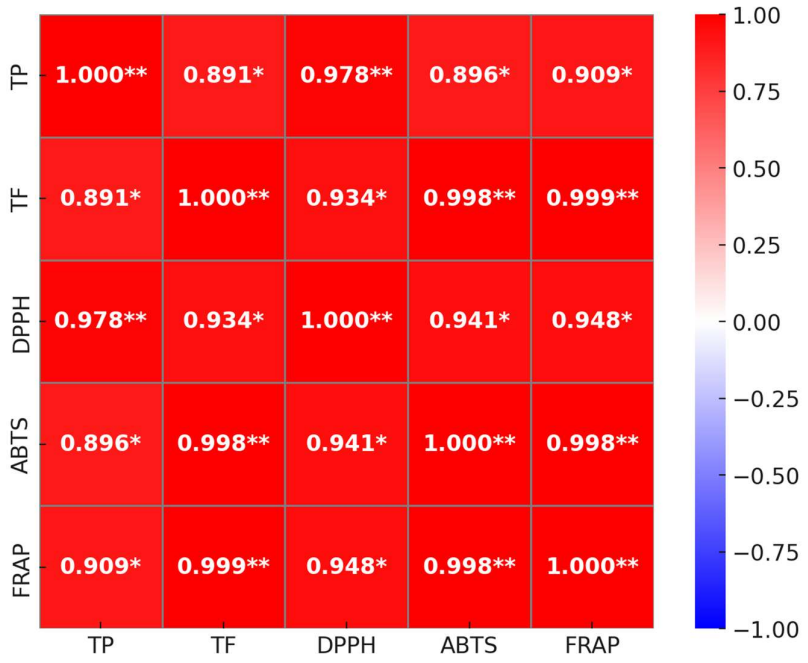


Figure 3. Heatmap illustrating correlation coefficients among total phenolic (TP) content, total flavonoid (TF) content, DPPH radical-scavenging activity (DPPH), ABTS radical-scavenging activity (ABTS), and ferric-reducing antioxidant power (FRAP). Blue and red shades represent negative and positive correlations between parameters. Statistically significant correlation differences are denoted by white asterisk, * $p < 0.05$, and ** $p < 0.01$.

4. Discussion

We confirmed, using HPLC-PDA, that the barley sprouts contained 8.14 ± 0.02 mg/g of saponarin (Table 3). According to Yoon et al. [28], extracts from two cultivars of another staple crop, Triticum aestivum (wheat), contained trace amounts of saponarin. Another study reported that three mature wheat varieties contained approximately 2.5 nmol of saponarin/seed [29]. As for other crops, the saponarin content was approximately 0.99 mg/g in dried leaf extracts of *Bryonia alba* L. [30], while *Hibiscus syriacus* L. (Rose of Sharon) was found to accumulate up to 1.528 mg/cm^2 of TFs in its flowers, with saponarin identified as the major component, accounting for more than 50% [31]. Kim et al. [20] measured the saponarin content in barley sprout extracts cultivated under various conditions using LED lighting and supplements, and reported a content range of 1.66–11.14 mg/g.

Previous studies on chlorophyll content in other crops have reported that rice seedlings contain approximately 2.4 mg/g of chlorophyll a and 0.9 mg/g of chlorophyll b [32]. The total chlorophyll content in green tea leaves was shown to be 4.33 ± 0.02 mg/g, while that of eucalyptus leaves was 1.26 mg/g [33]. Furthermore, the total chlorophyll content in broccoli was reported to be 6.94 mg/g [34], in spinach, 20.33 mg/g [35], and in mung bean, 1.85 mg/g [36]. Differences in chlorophyll content are likely because of various factors that influence the nutritional value of microgreens, such as seed type, growth conditions, nutrient solution components, and harvest timing [37].

According to Ra et al. [38], who studied the policosanol content of wheat seedlings based on the cultivation duration and variety, eicosanol, heneicosanol, and tricosanol were undetectable under all conditions. Additionally, the total policosanol content varied significantly, ranging from 0.58 to 9.91 mg/g, depending on cultivation time and variety. Similarly,

Lee et al. [39] measured the policosanol content of oat seedlings based on cultivar and cultivation period, and reported a total policosanol content ranging from 3.17 to 6.48 mg/g. No eicosanol, heneicosanol, tricosanol, heptacosanol, or triacontanol was detected. Compared to previous studies that examined policosanol content in other crops, barley sprouts contain unique compounds, such as eicosanol, heneicosanol, and tricosanol, which have not been detected in different crops.

Efforts to identify antioxidant compounds capable of scavenging reactive oxygen species (ROS) from food sources and natural products capable of scavenging ROS have long been actively pursued. Antioxidant activity is a multifaceted process involving various mechanisms, and is influenced by multiple factors, making it difficult to fully characterize using a single analytical approach [40]. Consequently, employing multiple antioxidant capacity assays is crucial to comprehensively assess the diverse mechanisms of antioxidant action [41]. In this study, the antioxidant capacity and phenolic content of barley sprouts were evaluated, and a correlation analysis demonstrated a strong positive relationship among the applied methods. These findings demonstrate that each analytical method is suitable for evaluating the antioxidant capacity of barley sprouts.

5. Conclusions

In this study, we aimed to evaluate the bioactive compounds and antioxidant capacity of barley sprouts by analyzing their saponarin, chlorophyll, and policosanol contents, as well as their TP and TF contents. Additionally, we assessed their antioxidant capacity using ABTS, DPPH, and FRAP assays, and performed a correlation analysis to validate the reliability of these methods. Our findings confirm that barley sprouts contain significant amounts of bioactive compounds, with 8.14 $\pm$ 0.02 mg/g of saponarin, 15.36 $\pm$ 0.18 mg/g of total chlorophyll, and 396.99 mg/100 g of policosanols. The IC_{50} values for antioxidant activity followed the sequence FRAP > DPPH > ABTS. Furthermore, strong positive correlations ($R > 0.891$, $p < 0.05$) among the different antioxidant assays support the reliability of these methods in assessing the antioxidant properties of barley sprouts. These results indicate that barley sprouts could be valuable functional ingredients, rich in antioxidants and bioactive compounds. Given the increasing demand for natural antioxidants and functional foods, barley sprouts can be a health-promoting ingredient in food formulations. Nevertheless, as this study was confined to in vitro analyses, additional investigations utilizing in vivo models and clinical trials are required to gain a more comprehensive understanding of the physiological effects of barley sprout consumption. In addition, investigating the influence of cultivation conditions, extraction methods, and processing techniques could further optimize the bioavailability and functional efficacy of barley sprout-derived antioxidants.

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