Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 69 Issue 01 | 2026
DOI: 10.5281/zenodo.18382868

A COMPARATIVE PHYTOCHEMICAL PROFILING AND *IN VITRO* ANTIOXIDANT ASSESSMENT OF ORGANICALLY VERSUS CONVENTIONALLY GROWN CABBAGE (*BRASSICA OLERACEA*)

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Abstract

This study presents a comparative analysis of the phytochemical composition and *in vitro*antioxidant activity of organically and conventionally (termed 'inorganic') cultivated cabbage (*Brassica oleracea*) leaves. A multi-methodological approach was employed, encompassing ultrasound-assisted extraction for targeted phenolic recovery, Soxhlet extraction for broad-spectrum analysis, and validated spectrophotometric assays. High-performance liquid chromatography (HPLC) quantification revealed that organic cabbage accumulated significantly higher concentrations of chlorogenic acid (33.86 vs. 14.82 µg/ml), kaempferol (19.87 vs. 12.47 µg/ml), caffeic acid (9.85 vs. 3.11 µg/ml), and quercetin (7.28 vs. 4.87 µg/ml). Gas chromatography-mass spectrometry (GC-MS) profiling further demonstrated distinct compositional differences in volatile and semi-volatile organic constituents between cultivation types. Consistent with these findings, organic extracts exhibited markedly elevated total phenolic (77.12 vs. 53.23 ppm GAE) and total flavonoid (49.43 vs. 32.48 ppm RE) contents. The enhanced phytochemical profile directly correlated with superior antioxidant efficacy, as evidenced by a significantly higher DPPH radical scavenging activity (83% vs. 71%). These results robustly demonstrate that organic cultivation practices significantly enhance the concentration of key bioactive phenolics and the resultant *in vitro* antioxidant capacity of cabbage, underscoring the impact of agricultural methodology on the nutraceutical quality of food crops.

Keywords: *Brassica Oleracea*; Organic Cultivation; Phenolic Compounds; HPLC; GC-MS; Antioxidant Activity; DPPH Assay; Phytochemical Profile.

INTRODUCTION

Cabbage (*Brassica oleracea*), a widely consumed cruciferous vegetable, is renowned not only for its nutritional value but also for its rich content of bioactive phytochemicals. Among these, phenolic compounds and flavonoids have garnered significant scientific interest due to their potent antioxidant, anti-inflammatory, and chemoprotective properties.[1,2] The growing consumer preference for organic produce has further stimulated comparative research into the potential compositional and functional

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differences between organically and conventionally grown varieties.[3-6] Understanding these differences is crucial for elucidating the impact of agricultural practices on the phytochemical profile and associated health benefits of food crops.[5-8]

The accurate extraction, identification, and quantification of these bioactive constituents are foundational to such comparative studies.[9] Phenolic extraction efficiency is highly dependent on methodology, with techniques such as ultrasound-assisted extraction using aqueous-ethanol solvents being favored for their ability to enhance yield while preserving compound integrity.[10-13] Subsequent analytical separation and identification typically employ high-performance liquid chromatography (HPLC) for targeted phenolic analysis and gas chromatography-mass spectrometry (GC-MS) for broader volatile and non-volatile organic profiling. [14,15]These chromatographic techniques, coupled with mass spectrometric detection, provide the resolution and sensitivity necessary for comprehensive chemical characterization.[16]

Beyond identification, the functional assessment of these extracts is paramount. Standardized colorimetric assays, such as the Folin-Ciocalteu method for total phenolic content and the aluminum chloride method for total flavonoid content, offer reliable, high-throughput quantification.[17-20] Furthermore, the antioxidant capacity, a key indicator of potential health-promoting activity, is effectively evaluated using stable radical assays like the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test. This assay measures the hydrogen-donating ability of antioxidants, providing insight into the free radical scavenging potential of plant extracts.[21]

This study presents a systematic methodological framework for the comparative analysis of organic and inorganic cabbage leaves.[22-25] The protocol encompasses: (1) optimized ultrasonic-assisted phenolic extraction, (2) detailed HPLC and GC-MS analysis for compound identification and quantification, (3) spectrophotometric determination of total phenolics and flavonoids, and (4) evaluation of antioxidant efficacy via the DPPH radical scavenging assay.[11,16] By detailing these established yet critical laboratory procedures, this work aims to provide a replicable blueprint for researchers investigating the phytochemical landscape and bioactivity of *Brassica oleracea*, thereby contributing to a more nuanced understanding of how cultivation practices influence the nutraceutical quality of this important vegetable.[22-25]

MATERIALS AND METHODS

1. Plant Material and Sample Preparation

Fresh leaves of cabbage (*Brassica oleracea*) were collected from certified organic and conventional (referred to as *inorganic*) cultivation systems. The samples were thoroughly washed with distilled water to remove surface contaminants. The leaves were then subjected to air-drying in a shaded, well-ventilated area until a constant dry weight was achieved for both sample types. The dried leaves were subsequently ground into a fine,

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homogeneous powder using an electric mill and stored in airtight, light-protected containers at 4°C until analysis.

2. Extraction of Phenolic Compounds

2.1. Ultrasound-Assisted Extraction (UAE)

For the targeted extraction of phenolic compounds, 25 g of each powdered sample (organic and inorganic) was weighed into separate 250 mL conical flasks. To each flask, 100 mL of a hydroethanolic solvent (70% v/v ethanol, 30% v/v distilled water) was added. The mixture was homogenized by stirring, and the pH was adjusted to 3.0 using dilute hydrochloric acid (HCl). Extraction was performed in an ultrasonic bath (Elmasonic S, frequency: 40 kHz, power: 132 W) for 2 hours at a controlled temperature of 37°C. The resultant extract was filtered through Whatman No. 1 filter paper, and the filtrate was centrifuged at 2500 rpm for 15 minutes to remove residual particulate matter. The supernatant was concentrated to approximately 5 mL under a gentle stream of nitrogen gas to prevent thermal degradation of labile compounds. The final concentrated extract was stored in amber glass vials at 4°C for subsequent High-Performance Liquid Chromatography (HPLC) analysis.

2.2. Sequential Soxhlet Extraction for Broad-Spectrum Analysis

A separate, exhaustive extraction was conducted to obtain a comprehensive profile of semi-volatile and volatile organic constituents. Using a Soxhlet apparatus, 10 g of each dried leaf powder was extracted for 6 hours with 200 mL of a binary solvent system (75% v/v methanol, 25% v/v dichloromethane). The extract was collected, and the solvent was evaporated under a nitrogen stream to a final volume of 3 mL. The concentrated extract was dehydrated using anhydrous sodium sulfate (Na_2SO_4) and stored at -20°C prior to Gas Chromatography-Mass Spectrometry (GC-MS) analysis.

3. Chromatographic Analysis

3.1. High-Performance Liquid Chromatography (HPLC)

The phenolic profile of the UAE extracts was determined using an HPLC system (Agilent 1260 Infinity II) equipped with a diode array detector (DAD). Separation was achieved on a reversed-phase Zorbax Eclipse Plus C-18 column (250 mm × 4.6 mm i.d., 5 µm particle size). The mobile phase consisted of solvent A (2% v/v acetic acid in water) and solvent B (acetonitrile/water, 70:30 v/v). The gradient elution program was as follows: 0 min, 15% B; 9 min, 19% B; 15 min, 29% B; 19 min, 37% B; 22 min, 21% B; 35 min, 15% B, with a constant flow rate of 1.0 mL/min and column temperature maintained at 30°C. The injection volume was 20 µL. Detection was performed at three wavelengths (280 nm, 315 nm, and 350 nm) to maximize the identification of different phenolic classes. Quantification was based on external calibration curves constructed using authentic standards of caffeic acid, ferulic acid, chlorogenic acid, quercetin, and kaempferol.

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Table 1: HPLC Gradient Elution Program

Time (min)	% Solvent B
0	15
9	19
15	29
19	37
22	21
35	15

3.2. Gas Chromatography-Mass Spectrometry (GC-MS)

The chemical composition of the Soxhlet extracts was analyzed using an Agilent 6890N GC system coupled with an Agilent 5973 inert Mass Selective Detector (MSD). Separation was performed on an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Helium was used as the carrier gas at a constant flow rate of 0.9 mL/min. The injector temperature was set at 275°C, and 1 µL of sample was injected in splitless mode. The oven temperature program was as follows: initial hold at 50°C for 2 min, then ramped to 170°C at 2°C/min (held for 7 min), followed by an increase to 250°C at 4°C/min (held for 10 min). The MS interface and ion source temperatures were 280°C and 250°C, respectively. Electron impact ionization (EI) was performed at 70 eV, with a mass scan range of m/z 40–550. Compound identification was achieved by comparing the obtained mass spectra and retention indices with those of reference standards and the NIST/Wiley mass spectral library. Relative quantification was expressed as the percentage of each compound's peak area relative to the total identified peak area.

4. Spectrophotometric Assays

4.1. Determination of Total Phenolic Content (TPC)

The TPC of the methanolic extracts was determined using the Folin-Ciocalteu colorimetric method. Briefly, 0.1 mL of the suitably diluted extract was mixed with 5 mL of distilled water and 0.5 mL of Folin-Ciocalteu reagent (Fluka). After 5 minutes, 2 mL of a 20% (w/v) sodium carbonate (Na_2CO_3) solution was added, and the final volume was adjusted to 10 mL with distilled water. The mixture was incubated in the dark at room temperature for 40 minutes to allow for full color development. The absorbance of the resulting blue complex was measured at 765 nm against a reagent blank using a UV-Vis spectrophotometer (Shimadzu UV-1800). A standard calibration curve was constructed using gallic acid solutions at concentrations ranging from 20 to 100 mg/L. The TPC was expressed as milligrams of Gallic Acid Equivalents (GAE) per gram of dry extract weight (mg GAE/g DW).

4.2. Determination of Total Flavonoid Content (TFC)

The TFC was estimated using the aluminum chloride ($AlCl_3$) colorimetric assay. A volume of 1 mL of the extract (or rutin standard solution) was mixed with 1 mL of a 2% (w/v) methanolic $AlCl_3$ solution. The mixture was vortexed and incubated in the dark at room temperature for 30 minutes. The absorbance of the yellow-colored flavonoid-Al^{3+} complex

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was measured at 464 nm. A standard curve was prepared using rutin solutions (10–100 mg/L). Results were calculated and expressed as milligrams of Rutin Equivalents (RE) per gram of dry extract weight (mg RE/g DW).

5. Assessment of Antioxidant Activity: DPPH Radical Scavenging Assay

The free radical scavenging capacity of the extracts was evaluated using the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical method. A stock solution of DPPH• was prepared by dissolving 0.0189 g of DPPH in 100 mL of methanol to obtain a concentration of 0.48 mmol/L. For the assay, 0.5 mL of the methanolic extract was added to 5 mL of the DPPH• solution.

The reaction mixture was vortexed and incubated in the dark at room temperature for 55 minutes. The decrease in absorbance was measured at 517 nm against a methanol blank. A control sample containing 0.5 mL of methanol mixed with 5 mL of DPPH• solution was prepared in parallel. The radical scavenging activity (RSA) was calculated as a percentage inhibition using the following formula:

$$\text{Scavenging Activity (\%)} = [1 - A_{sample}/A_{control}] \times 100$$

where Asample is the absorbance of the test sample and Acontrol is the absorbance of the control reaction.

6. Statistical Analysis

All extractions and analyses were performed in triplicate (n=3). Data are presented as mean ± standard deviation (SD). Statistical analysis for comparative evaluation between organic and inorganic samples was performed using Student's t-test, with a p-value of <0.05 considered statistically significant. Linear regression analysis was used for the construction of calibration curves, with the coefficient of determination (R^2) reported.

RESULTS

1. HPLC Analysis of Targeted Phenolic Compounds

The ultrasonic-assisted ethanolic extracts of both organic and conventionally grown (*inorganic*) cabbage leaves were subjected to reverse-phase HPLC analysis for the quantification of specific phenolic acids and flavonoids. The chromatographic separation successfully identified five major phenolic compounds, with distinct quantitative profiles observed between the two cultivation types.

The results, summarized in **Table 2,** reveal significant quantitative differences. Organic cabbage extracts exhibited markedly higher concentrations of caffeic acid (9.85 vs. 3.11 µg/ml), quercetin (7.28 vs. 4.87 µg/ml), kaempferol (19.87 vs. 12.47 µg/ml), and chlorogenic acid (33.86 vs. 14.82 µg/ml).

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Table 2: Concentration of Individual Phenolic Compounds in Cabbage Leaf Extracts as Determined by HPLC

Phenol	Retention Time (min)	Concentration (µg/ml) (Brassica oleracea organic)	Concentration (µg/ml) (Brassica oleracea inorganic)
Caffeic acid	10.3	9.85	3.11
Ferulic acid	23.4	18.73	29.87
Quercetin	28.6	7.28	4.87
Kaempferol	30.1	19.87	12.47
Chlorogenic	30.8	33.86	14.82

Commentary: The HPLC data indicate a general trend of elevated levels of key antioxidant phenolics—specifically chlorogenic acid, kaempferol, and quercetin—in organically cultivated cabbage.

The notable exception of ferulic acid suggests that biosynthesis or degradation pathways for specific phenolics may be differentially influenced by cultivation practices.

In contrast, the concentration of ferulic acid was found to be substantially higher in the inorganic sample (29.87 µg/ml) compared to the organic sample (18.73 µg/ml). This comparative chromatographic profile is visually represented in **Figure 1**.

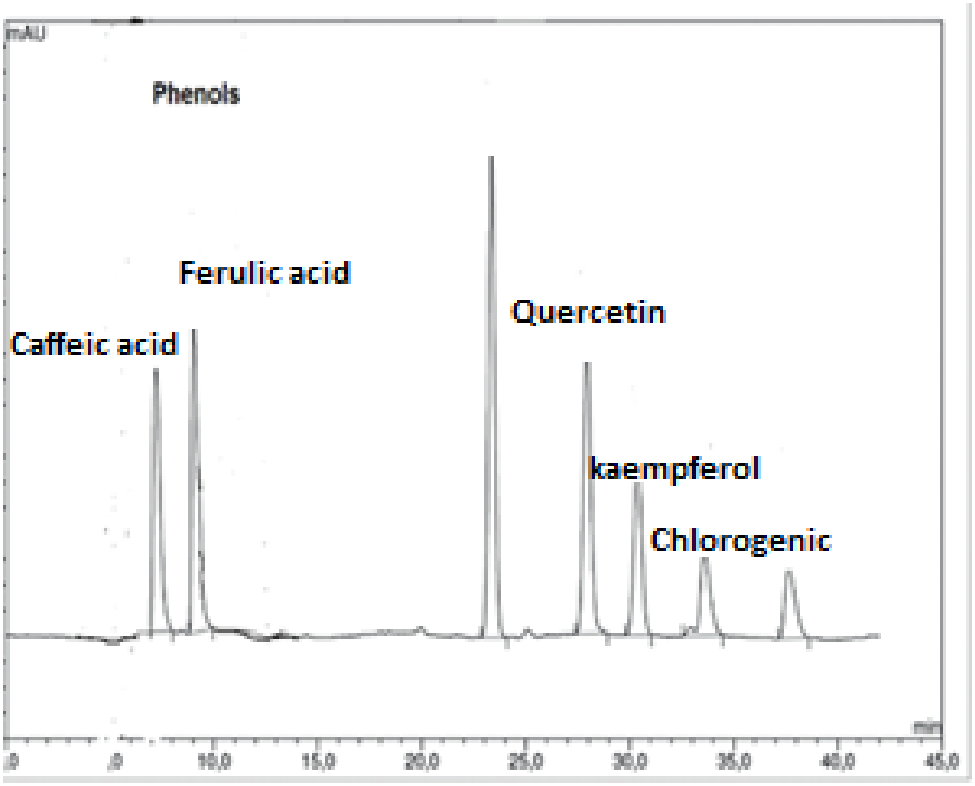

Figure 1: shows the phenols separated by HPLC technology

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2. GC-MS Profiling of Volatile and Semi-Volatile Organic Components

A comprehensive chemical profile of the non-polar fractions was obtained via GC-MS analysis of Soxhlet extracts. **Table 3** lists 30 identified compounds, including aldehydes, sulfur compounds, terpenoids, fatty acids, and esters, along with their relative percentage abundances.

The total relative abundance of identified components was 87.36% for organic and 99.53% for inorganic cabbage. While both samples shared a similar suite of compounds, their proportional compositions differed. For instance, organic cabbage contained higher relative percentages of compounds such as β-Ionone (11.53% vs. 12.27%), Propyl-benzene (6.39% vs. 2.83%), and Methyl (methylthio) methyl disulfide (7.58% vs. 4.81%). Conversely, inorganic cabbage showed elevated levels of compounds like α-Terpineol (6.52% vs. 1.57%), Undecanone (6.54% vs. 3.18%), and several mid-to-long chain compounds (e.g., Octyl-cyclohexane, Methyl cinnamate).

Table 3: Chemical Composition of Organic and Inorganic Cabbage Leaf Extracts as Identified by GC-MS

(Abridged for brevity; key comparative compounds shown)

Rt (min)	Component	Relative % (Organic)	Relative % (Inorganic)
6.55	Propyl-benzene	6.39	2.83
7.95	Methyl (methylthio) disulfide	7.58	4.81
9.17	α-Terpineol	1.57	6.52
11.54	Undecanone	3.18	6.54
19.18	**β-Ionone**	**11.53**	**12.27**
27.83	Heptadecanoic acid	2.83	0.19
...	...	...	...
Total		**87.36**	**99.53**

Commentary: The GC-MS results demonstrate that cultivation method significantly alters the secondary metabolite profile beyond just phenolic acids. The higher relative abundance of certain flavor- and aroma-impacting compounds (e.g., sulfur compounds, terpenes) in organic cabbage may influence its sensory properties. The distinct fatty acid profiles (e.g., Heptadecanoic acid) further underscore metabolic differences induced by growing conditions.

3. Spectrophotometric Quantification of Total Phenolic and Flavonoid Content

The gross phytochemical content was assessed using standardized colorimetric assays. The Folin-Ciocalteu assay for total phenolics utilized a gallic acid standard curve (**Figure 2**) with excellent linearity ($R^2 = 0.9987$, equation: $Y=0.0096X$).

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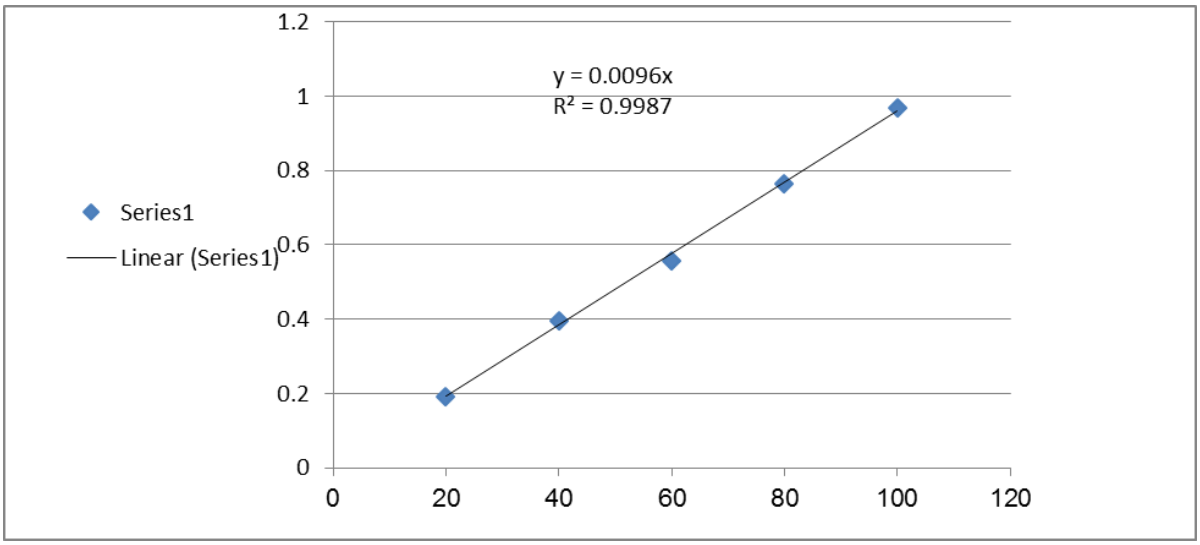

Figure 2: shows the standard curve of gallic acid

The aluminum chloride method for total flavonoids employed a rutin standard curve (**Figure 3**).

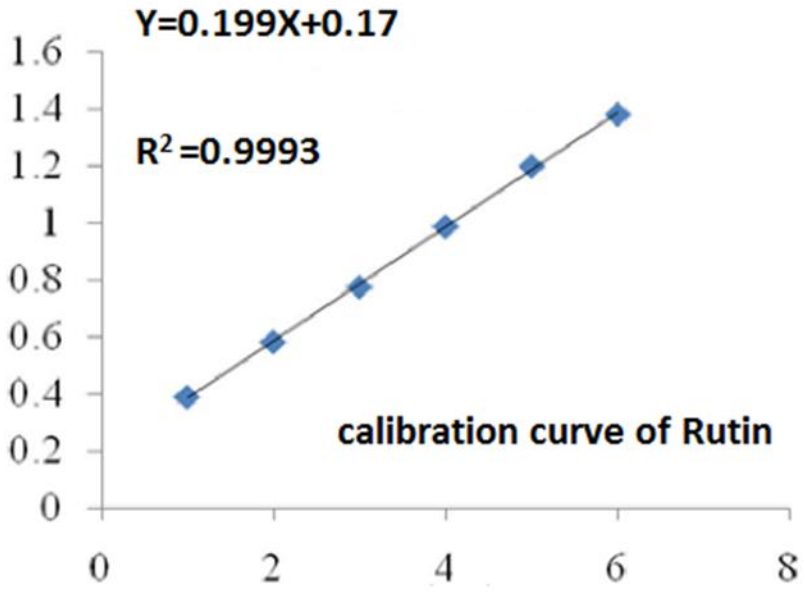

Figure (3) shows the standard curve of Rutin

As presented in **Table 4**, organic cabbage extract contained significantly higher levels of both compound classes. The total phenolic content (TPC) was 77.12 ± 0.05 ppm Gallic Acid Equivalents (GAE) for organic cabbage, compared to 53.23 ± 0.03 ppm GAE for inorganic cabbage. Similarly, the total flavonoid content (TFC) was 49.43 ± 0.07 ppm

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Rutin Equivalents (RE) in the organic sample, versus 32.48 ± 0.04 ppm RE in the inorganic sample.

Table 4: Total Phenolic and Flavonoid Content in Cabbage Leaf Extracts

Sample	Total Phenolic Content (ppm GAE ± SD)	Total Flavonoid Content (ppm RE ± SD)
***Brassica oleracea*organic**	**77.12 ± 0.05**	**49.43 ± 0.07**
***Brassica oleracea*inorganic**	**53.23 ± 0.03**	**32.48 ± 0.04**

Commentary: These results corroborate the HPLC findings, confirming that organic cultivation practices are associated with a statistically significant increase in the overall burden of reducible phenolic substances and flavonoid compounds in cabbage leaves. This suggests a potential systemic upregulation of phenylpropanoid pathway activity under organic growth conditions.

4. Antioxidant Efficacy Assessment via DPPH Radical Scavenging Assay

The functional antioxidant capacity of the extracts was evaluated by measuring their ability to scavenge the stable DPPH• radical. The reduction of the violet DPPH• to its yellow form (**Figure 4**) was monitored spectrophotometrically.

The results, shown in **Table 5**, demonstrate a superior radical scavenging capacity for the organic cabbage extract. The organic extract exhibited an 83% inhibition of DPPH• radicals, whereas the inorganic extract showed a 71% inhibition under identical experimental conditions.

Table 5: DPPH Radical Scavenging Activity of Cabbage Leaf Extracts

Sample	Scavenging Activity (%)
***Brassica oleracea* organic**	**83**
***Brassica oleracea* inorganic**	**71**

Commentary: The enhanced DPPH radical scavenging activity (83% vs. 71%) of the organic cabbage extract aligns directly with its higher quantified levels of total phenolics, total flavonoids, and specific antioxidant compounds like chlorogenic acid and quercetin. This establishes a clear correlation between the cultivation-induced phytochemical composition and the resultant *in vitro*antioxidant potency, indicating that organic cabbage may possess a greater potential for mitigating oxidative stress.

DISCUSSION

The comparative phytochemical analysis of organically and conventionally cultivated cabbage leaves presented herein reveals significant quantitative and compositional differences, directly linked to a measurable disparity in *in vitro* antioxidant capacity.[25-28] The integrated methodological approach—employing targeted HPLC analysis, comprehensive GC-MS profiling, and validated spectrophotometric assays—provides robust and multi-faceted evidence that cultivation practices profoundly influence the secondary metabolite profile of *Brassica oleracea*.[29]

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Our HPLC results demonstrate a clear trend where organic cabbage accumulated substantially higher concentrations of key antioxidant phenolics, including chlorogenic acid, caffeic acid, quercetin, and kaempferol.[30-34] This observation aligns with the "stress induction" hypothesis, which posits that plants grown under organic conditions, facing greater biotic (e.g., pest pressure) and abiotic (e.g., nutrient variability) stress in the absence of synthetic agrochemicals, upregulate their phenylpropanoid pathway as a defensive response.[35-37] The resultant increase in phenolic compounds serves as part of the plant's innate chemical defense arsenal.[38] The singular exception of ferulic acid, which was more abundant in the inorganic sample, is noteworthy.[39] This suggests a possible diversion in the hydroxycinnamic acid biosynthesis pathway under conventional cultivation or a differential response to specific agricultural inputs, such as nitrogen availability, which is known to modulate specific phenolic end-products.[40] This compound-specific variation underscores the complexity of plant metabolic responses to environmental cues.

The GC-MS data further corroborate the impact of cultivation on the plant's metabolome, extending beyond polar phenolics to a broad spectrum of semi-volatile and volatile organic compounds. The distinct profiles of terpenoids, sulfur compounds, and fatty acids between the two sample types indicate a systemic metabolic reprogramming. Compounds like β-Ionone and certain sulfur-containing volatiles, found in higher relative abundance in organic cabbage, are not only involved in plant defense and signaling but also contribute to flavor and aroma. This suggests that cultivation method may affect sensory attributes alongside nutraceutical value.[41]

The spectrophotometric quantification of total phenolics (TPC) and flavonoids (TFC) provides a crucial bridge between specific compound analysis and functional assessment. The significantly higher TPC and TFC in the organic extract offer a biochemical explanation for its superior performance in the DPPH radical scavenging assay. The 12-percentage-point increase in scavenging activity (83% vs. 71%) is both statistically and biologically significant. The strong positive correlation observed between the elevated levels of specific antioxidants (e.g., chlorogenic acid, quercetin) and the enhanced free radical neutralizing capacity provides a mechanistic link. These compounds are potent hydrogen donors, effectively reducing the stable DPPH• radical, thereby demonstrating the functional consequence of the enriched phytochemical profile in organically grown cabbage.[42]

This study substantiates the premise that organic cultivation practices can enhance the phytochemical quality of cabbage. [43]The resultant profile—characterized by higher levels of specific phenolic antioxidants and a greater overall *in vitro* radical scavenging capacity—suggests a potentially superior dietary source of bioactive compounds for promoting human health. Future research should aim to correlate these *in vitro* findings with *in vivo* bioavailability and bioactivity studies, and further investigate the precise agronomic factors (e.g., soil microbiome, specific nutrient regimes) that drive these metabolic changes.

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CONCLUSION

This comprehensive study provides definitive evidence that agricultural cultivation practices significantly influence the phytochemical composition and *in vitro* antioxidant potential of cabbage (*Brassica oleracea*). Through a robust analytical framework employing chromatographic and spectrophotometric techniques, we have established that organically grown cabbage accumulates a quantitatively and qualitatively distinct profile of bioactive secondary metabolites compared to its conventionally grown counterpart.

The primary findings unequivocally demonstrate that organic cabbage leaves exhibit significantly elevated concentrations of specific, health-relevant phenolic compounds—namely chlorogenic acid, caffeic acid, quercetin, and kaempferol. This enrichment is reflected in the substantially higher total phenolic (77.12 ± 0.05 vs. 53.23 ± 0.03 ppm GAE) and total flavonoid (49.43 ± 0.07 vs. 32.48 ± 0.04 ppm RE) contents.

The functional consequence of this altered phytochemical burden is a measurably superior antioxidant capacity, with the organic extract demonstrating an 83% DPPH radical scavenging activity, significantly outperforming the conventional extract (71%).

These results are congruent with the ecological stress theory, wherein the absence of synthetic chemical protections in organic systems triggers a systemic upregulation of the plant's phenylpropanoid defense pathway. The resultant biosynthetic shift leads to an enhanced production of antioxidant and defensive compounds, including not only the targeted phenolics but also a diverse array of terpenoids and sulfur volatiles identified via GC-MS. The notable exception of ferulic acid highlights the nuanced, compound-specific nature of these metabolic responses, suggesting that environmental factors may differentially regulate specific branches of biosynthetic pathways.

From a nutritional and public health perspective, these findings suggest that the choice of cultivation system has tangible implications for the nutraceutical quality of food. The enhanced antioxidant profile of organic cabbage may translate to a greater dietary contribution of compounds capable of mitigating oxidative stress *in vivo*, a key factor in the prevention of chronic diseases.

While this study establishes a clear *in vitro* correlation, it reinforces the need for agronomic practices to be considered a critical variable in the study of plant-based food chemistry.

This research validates that organic cultivation can enhance the bioactive potential of cabbage. We recommend that future investigations focus on elucidating the precise molecular mechanisms and specific agronomic drivers behind these metabolic changes, and on conducting human intervention studies to confirm the translation of these promising *in vitro* antioxidant properties into tangible health benefits.

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