Pharmacogn J. 2025; 17(1): 58-62
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Research Article

Effect of Different Extraction Solvents on the Total Phenolic Content and Antioxidant Activity of *Brassica oleracea* var. *italica*

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ABSTRACT

This study offers an alternative solution for the prevention and/or treatment of diseases caused by free radicals. The objective was to evaluate the effect of different solvents on the total phenolic content and antioxidant activity of *Brassica oleracea* var. *italica* (broccoli). **Methods:** The sample, consisting of stems and florets of *Brassica oleracea var. italica* (broccoli), were collected from the Chocas community in Carabayllo, Lima, Peru. Three extracts were prepared using different solvents, including a mixture of ethanol and water. The total phenolic content was determined using the Folin-Ciocalteu method, and antioxidant capacity was evaluated using two specific assays (DPPH and ABTS). Additionally, the correlation between total phenolic content and antioxidant activity was analyzed. **Results:** The hydroethanolic extract demonstrated the highest phenolic content, with 686.02 mg GAE/100 g dry matter). It also exhibited strong antioxidant activity, measuring 1035.81 mg TE/100 g DM in the DPPH assay and 6506.94 mg TE/100 g DM in the ABTS assay. **Conclusion:** The highest total phenolic content and antioxidant activity of *Brassica oleracea* var. *italica* were found in the hydroethanolic extract, which showed a significant high correlation. Frequent consumption of broccoli in the diet is recommended due to its high values.
Keywords: Antioxidant, Broccoli, Extract, Polyphenol, Vegetable.

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History

- Submission Date: 01-12-2024;
- Review completed: 26-12-2024;
- Accepted Date: 06-01-2025.

DOI : 10.5530/pj.2025.17.7

Article Available online

http://www.phcogj.com/v17/i1

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INTRODUCTION

Free radicals are unstable atoms produced by redox reactions within the body. These atoms possess an unpaired electron in their atomic configuration and exhibit a high oxidation potential, targeting lipids, proteins, and nucleic acids.[1] Due to their mobility throughout the human body, they seek to extract electrons to achieve stable molecular forms. When they interact with biological molecules and tissues, they transform into toxic chemicals that cause oxidative damage and tissue dysfunction.[2] These are major contributors to the development and progression of various diseases.[3]

Fortunately for human health, free radicals can be neutralized by antioxidants due to their ability to donate electrons, stabilizing these reactive molecules. Antioxidants provide multiple health benefits, preventing conditions such as inflammation, allergies, thrombosis, viral infections, and even cancer.[4] These compounds can be found in foods, and the search for such sources has become a growing trend.[5]

Among the elements that similarly benefit health are phenolic compounds, which are abundant in plant-based foods. These are referred to as polyphenols when they contain more than one phenolic group. These compounds are conjugated with one or more sugar residues, such as monosaccharides, disaccharides, or even oligosaccharides, with glucose being the most common.[6] They exhibit antioxidant, anti-inflammatory, antimicrobial, and anticancer properties.[7] The use of various polyphenols and polyenes derived from fruits and vegetables protects proteins, lipids, and DNA from free radical-induced damage.[3]

One of the most notable foods containing these substances is *Brassica oleracea* var. *italica*, commonly known as broccoli. Studies have shown that broccoli contains vitamins, fiber, glucosinolates, and phenolic compounds in its raw matrix,[8] which have the potential to eradicate tumor cells and reduce the risk of developing cancer. Therefore, this vegetable may be key to controlling chronic diseases, such as metabolic syndrome.[7]

Research has demonstrated that the optimization of bioactive components in broccoli pulp via double-drum drying achieved a 147.6% increase, indicating this method as the most suitable for enhancing total phenolic content in dehydrated broccoli.[9] Additionally, peptides from the stems and leaves of *Brassica oleracea* var. *italica* at a concentration of 5.0 mg/mL showed a 72.8% radical scavenging rate, comparable to glutathione at 1.0 mg/mL, making it a promising functional food with high nutritional value for human health.[10]

Furthermore, florets from six genotypes of broccoli at three different inflorescence development stages were reported to contain 470.05 mg GAE/100 g dry matter (DM)[11]. In a separate organic improvement study, an average of 309.100 mg GAE/100 g was observed after analyzing 52 genotypes of *Brassica oleracea L.* var. *italica* (broccoli) and *Brassica oleracea L.* var. *botrytis* (cauliflower).[12] Similarly, across 13 seed types, total phenolic content ranged from 469.000 to 1321.000 mg GAE/100 g DM, with antioxidant activity varying between 830.000 and 960.000 mg TE/100 g DM.[13]

Phenolic compounds are key antioxidants for preventing oxidative damage associated with chronic diseases[14] and can be obtained by consuming *Brassica oleracea* var. *italica*, commonly referred to as broccoli. Given its significance, optimizing the extraction process is essential, as efficiency depends

Cite this article: Florian VNE, Sisniegas CGM, Valdiviezo-Campos JE. Effect of Different Extraction Solvents on the Total Phenolic Content and Antioxidant Activity of *Brassica oleracea* var. *italica*. Pharmacogn J. 2025;17(1): 58-62.

on the solvent used. Identifying the most suitable solvent can maximize the phenolic content and antioxidant activity of the extract. This knowledge has practical applications in the food, pharmaceutical, and supplement industries, promoting high-quality functional products. Therefore, this study aimed to evaluate the effect of different extraction solvents on the total phenolic content and antioxidant activity of extracts from *Brassica oleracea* var. *italica* (broccoli).

MATERIAL AND METHODS

Reagents and solvents

The reagents and solvents used included 96° GL ethanol (CKF®), distilled water (Dropaksa®), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and Folin-Ciocalteu reagent (Sigma Aldrich®). Sodium bicarbonate and sodium acetate (Merck®) were employed, with gallic acid (Merck®) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, Sigma Aldrich®) as standards.

Botanical Material

The species used was *Brassica oleracea* var. *italica*, collected from the Chocas population center in the Carabayllo district, located at an altitude of 470 meters above sea level (m.a.s.l.) with geographic coordinates 11°51'00"S and 77°02'00"W, in the province and department of Lima, Peru.

Taxonomic Identification

A complete specimen was pressed and prepared following the standard protocols of the *Herbarium Truxillense*. It was taxonomically identified and registered under code N° 60829.

Preparation and Extraction

Two kilograms of *B. oleracea* var. *italica* were collected, selecting stems and florets as the plant material, ensuring their integrity and absence of inert material or decomposition. The plant material was shade-dried and then further dried in a oven (Memmert) at 50 °C for five days. The dried material was mechanically milled using a Corona Mill to produce fine particles. Three extraction systems (5% w/v) were prepared using 96° ethanol, 70° ethanol, and water. The solvents were selected based on their increasing polarity, considering their impact on preserving antioxidant activity. Additionally, the extract is safe and unlikely to cause undesirable effects. The 5% w/v ratio provides an optimal balance between solvent and sample, preventing saturation while ensuring efficient and reproducible extraction. The mixtures were agitated at 400 rpm for 60 minutes at 100 °C using a magnetic stirrer with a hot plate (Ahn). Afterward, the mixtures were filtered and stored at 6 °C for subsequent use.

Total Phenolic Content

The total phenolic content was determined using the Folin-Ciocalteu method,[15,16] with slight modifications. A calibration curve was prepared with gallic acid at concentrations ranging from 10 to 100 µg/mL. For each standard solution, 2 mL of 10 % Folin-Ciocalteu reagent, 4.4 mL of 7.5% sodium bicarbonate, and distilled water were added to a final volume of 10 mL. For the test samples, 0.4, 0.2, and 0.2 mL of ethanolic, hydroethanolic, and aqueous extracts, respectively, were combined with 2 mL of 10% Folin-Ciocalteu reagent and 4.4 mL of 7.5% sodium bicarbonate, and the final volume was adjusted to 10 mL with distilled water. The mixtures were covered with aluminum foil and left in darkness for 60 minutes. Absorbance was measured at 765 nm using a Peack Instruments C-7000V spectrophotometer. The procedure was repeated in triplicate, and TPC was expressed as mg gallic acid equivalents (GAE) per 100 g of dry matter (DM).

Antioxidant Activity

DPPH Method

The antioxidant activity was evaluated using the DPPH radical scavenging assay,[17] with modifications. A 0.1 mM DPPH radical solution in 96° ethanol was prepared. A calibration curve was constructed with Trolox at concentrations ranging from 5 to 30 µM, adjusted to 10 mL with the DPPH solution. For the test samples, dilutions (3:10 v/v) were prepared. Then, 1 mL of each dilution was combined with 10 mL of DPPH solution. The mixtures were covered with aluminum foil and incubated in darkness for 45 minutes. Absorbance was measured at 517 nm using a Peack Instruments C-7000V spectrophotometer. This procedure was repeated in triplicate, and the results were expressed as mg Trolox per 100 g of DM.

ABTS Method

The ABTS assay[18] was conducted with modifications. An ABTS radical solution was prepared by mixing 7 mM ABTS and 2.45 mM potassium persulfate in a 50:50 ethanol-water mixture. The solution was incubated overnight in darkness at room temperature. A calibration curve was constructed with Trolox at concentrations ranging from 5 to 30 µM, adjusted to 10 mL with the ABTS solution. For the test samples, 0.3, 0.1, and 0.1 mL of ethanolic, hydroethanolic, and aqueous extracts, respectively, were combined with 10 mL of ABTS solution. The mixtures were covered with aluminum foil and incubated in darkness for 45 minutes. Absorbance was measured at 734 nm using a Peack Instruments C-7000V spectrophotometer. This procedure was repeated in triplicate, and results were expressed as mg Trolox per 100 g of DM.

Statistical Analysis

All experiments were performed in triplicate, and results are presented as descriptive graphs (mean ± standard deviation). Correlation analysis between TPC and antioxidant activity was conducted using Janovi 2.6.17, a free software. Statistical significance between groups was analyzed through one-way analysis of variance (ANOVA) using SPSS software version 27. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Figure 1 shows that the total phenolic content (TPC) is highest in the hydroalcoholic extract (70% ethanol), with a value of 686.020 mg GAE/100 g DM, while the lowest phenolic content was found in the ethanolic extract (96° GL ethanol), with 291.598 mg GAE/100 g DM. The data presented in Table 1 confirm that 70° GL ethanol was the most effective solvent, with statistically significant differences ($p < 0.01$) compared to the other solvents evaluated.

Figure 2 illustrates how the different extracts vary in their antioxidant capacity depending on the evaluation method used. When measuring antioxidant capacity against the DPPH radical, the aqueous extract exhibited the highest antioxidant activity, with 1035.806 mg TE/100 g DM. In contrast, against the ABTS radical, the hydroalcoholic extract showed superior activity, with a value of 6506.94 mg TE/100 g DM.

The data presented in Table 2 show that aqueous solvents and 70° GL ethanol were significantly more effective at extracting antioxidant compounds compared to 96° GL ethanol, highlighting the importance of solvent polarity. The differences observed between the solvents used are statistically significant ($p < 0.01$).

Figure 3 shows the correlation analysis between the total phenolic content (measured using the Folin-Ciocalteu method) and antioxidant activity (evaluated using DPPH and ABTS methods). A highly significant positive correlation was found between the antioxidant capacity measured by the ABTS method and the total phenolic content.

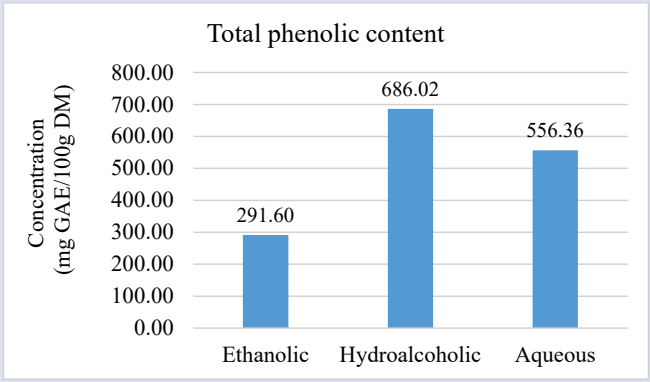

Figure 1: Total phenolic content in extracts of *B. oleracea* var. *italica.*

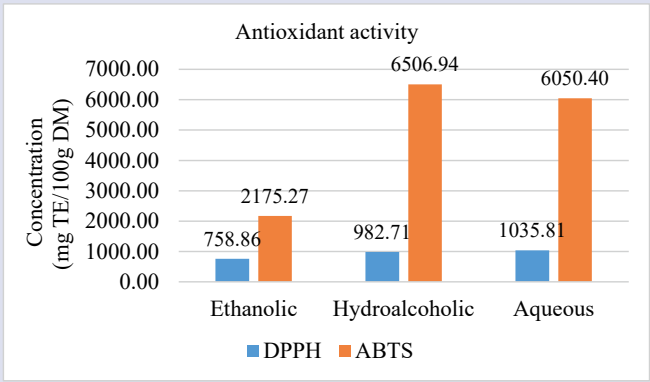

Figure 2: Antioxidant activity in extracts of *B. oleracea* var. *italica.*

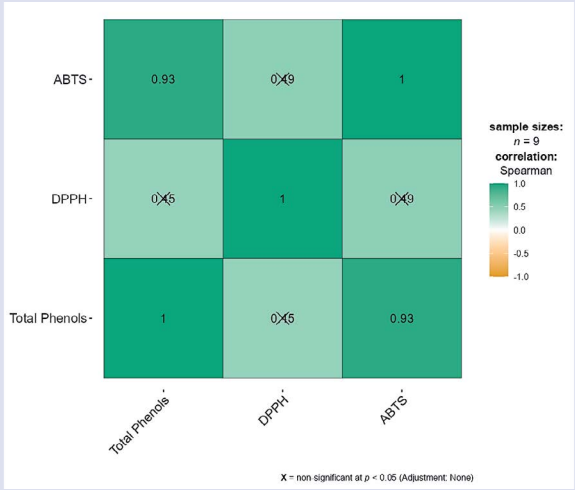

Figure 3: Correlation analysis between total phenolics and antioxidant activity of *B. oleracea* var. *itálica.*

Table 1: Concentration of total phenols in extracts of *B. oleracea* var. *itálica.*

Dissolvent	Concentration (mg GAE/ 100g DM)*	ANOVA Test (p)
Ethanol 96° GL	291,598 ± 0,00	
Ethanol 70° GL	686,020 ± 0, 56	<0,01
Water	556,360 ± 0,56	

Values are expressed as means ± standard errors (n=3)*; GAE: gallic acid equivalent; DM: dry matter.

Table 2: Antioxidant activity using DPPH and ABTS in extracts of *B. oleracea* var. *italica*.

Dissolvent	Concentration (mg ET/100g DM)*		ANOVA Test (p)
	DPPH	ABTS	
Ethanol 96° GL	758,864 ± 0,00	2175,27 ± 1,31	
Ethanol 70° GL	982,706 ± 0,00	6506,94 ± 0,00	<0,01
Water	1035,806 ± 1,865	6050,40 ± 7,80	

These results underscore the importance of phenolic compounds as the main contributors to antioxidant activity in *B. oleracea var. italica* extracts. The variability in antioxidant activity and total phenolic content depending on the solvent used highlights the importance of optimizing extraction conditions to maximize the yield and bioactive functionality of the extracted compounds.

DISCUSSION

In Table 1, the values for total phenolic content in the ethanolic, hydroalcoholic, and aqueous extracts are reported as 291.598, 686.020, and 556.360 mg GAE/100g of dry matter, respectively. Comparing the results, the hydroalcoholic extract exhibits a higher content than that obtained by Ram et al., who, while monitoring the total phenolic profile in florets of six broccoli genotypes using 80% methanolic extracts at three different stages of inflorescence development, found a concentration of 470.050 mg GAE/100g of dry matter.[11] This discrepancy may be due to the smaller amount of plant material used, which directly affects the presence of phenolic compounds. Additionally, the choice of plant material influences the concentration of bioactives, impacting the total phenolic content.[19]

Similarly, the concentration was lower in the study by Montaner et al., who, while evaluating the bioactive compound content in 13 types of broccoli seeds (*Brassica oleracea* var. *italica*) using 80% methanolic extracts, reported total phenolic contents ranging from 5.49 to 13.21 mg GAE/g of dry weight.[13] These outstanding values are possibly due to modifications in the quantification method and differences in the solvents used to more efficiently extract phenolic compounds.[20]

On the other hand, the total phenolic content investigated by Scalzo et al. was lower. They analyzed the total phenolic content in 52 hybrid genotypes of cauliflower (*B. oleracea* var. *botrytis*) and broccoli over four years and reported that extracts prepared with acidified ethanol exhibited a concentration of 309.100 mg GAE/100 g of dry material.[12] Additionally, the results for the hydroalcoholic and aqueous extracts also differ from those reported by Bhatt et al., who determined the phenolic content of *Brassica oleracea* var. *botrytis* in 50% ethanolic extracts at high and low temperatures, reporting values of 17.580 mg and 13.330 mg GAE/100 g, respectively.[21] Similarly, Afshari et al. reported a phenolic content of 131.92 mg GAE/g of dry broccoli matters (*B. oleracea* var. *italica*).[22]

Several authors argue that antioxidants act as electron donors, protecting cells from damage caused by free radicals.[22-25] Considering this, Table 2 presents the antioxidant activity determined by two methods (DPPH and ABTS). The antioxidant activity against the DPPH free radical in *B. oleracea* var. *italica* "broccoli" showed concentrations of 758.864, 982.706, and 1035.806 mg TE/100 g of dry matter for the ethanolic, hydroalcoholic, and aqueous extracts, respectively.

The antioxidant capacity of the ethanolic extract was lower compared to the study by Montaner et al., who evaluated the antioxidant activity in broccoli seeds (*B. oleracea* var. *italica*) using 80% methanolic extracts and reported values ranging from 83.00 to 96.00 mg TE/100 g of dry matter.[13] Similarly, Gudiño et al. found that DPPH and ABTS radical

scavenging activities varied by variety, with values ranging from 436 to 360 mg Trolox/100 g for DPPH and from 1631 to 843 mg Trolox/100 g for ABTS, respectively.[19]

This could explain why the volume used in each method and the solubility of methanol for bioactives enhance the desorption of antioxidants more efficiently in certain vegetable varieties.[20] Similarly, the antioxidant activity results for *B. oleracea* var. *italica* "broccoli" obtained using the ABTS method showed values of 2175.27, 6506.94, and 6050.40 mg TE/100 g of dry matter for the ethanolic, hydroalcoholic, and aqueous extracts, respectively. Among these, the ethanolic and aqueous extracts exhibited higher values compared to those reported by Uvaraj et al., who studied the antioxidant activity of fresh broccoli stems and florets. Using fine broccoli powder ground with liquid nitrogen, they prepared extracts with 70% methanol and sterile water, reporting approximately 3 mg and 4 mg TE/100 g of dry sample for the methanolic and aqueous extracts, respectively.[26]

On the other hand, Kaulmanm et al., in their study analyzing different *B. oleracea* varieties, reported 583 mg AAE/100 g of fresh matter.[27] Similarly, Othman et al. reported antioxidant activity ranging from 34.75 to 53.34 mg dihydroquercetin equivalents/g of fresh weight after soaking kale seeds (*B. oleracea* L. var. *acephala* DC.) in a solution based on amorphous silicon dioxide.[28]

Variations in total phenolic content and antioxidant activity were observed in the *B. oleracea* extracts depending on the solvent used for extraction, highlighting the influence of solvent type on these parameters. Previous studies support these findings, indicating a strong dependence of antioxidant activity on the solvent used.[20,24,29] The antioxidant activity of phenolic compounds varies according to the solvent's polarity, with polar extracts being more effective in exhibiting higher antioxidant activity.[30] Notably, the hydroethanolic extract of *B. oleracea* stands out as a promising source due to its ability to scavenge free radicals and mitigate oxidative stress.

A high positive correlation (R = 0.93) was found between antioxidant activity measured by ABTS and total phenolic content, as shown in Figure 3. Similar but lower results were reported by Rumpf et al., who conducted a statistical evaluation of the DPPH, ABTS, FRAP, and Folin-Ciocalteu assays and observed a moderate correlation between ABTS and FC (R = 0.757).[18] Similarly, Castañeda-Valbuena et al. studied the effect of ultrasound extraction conditions on the antioxidant capacity of mango by-product extracts and found a low positive correlation between ABTS and TPC (R = 0.471).[29]

The total phenolic content shows a correlation of 93% and 45% for ABTS and DPPH, respectively. The high positive correlation between antioxidant activity by ABTS (R = 0.93) and total phenolic content in *B. oleracea* indicates that polyphenols are the main contributors to its antioxidant capacity, as shown in Figure 3. This finding is consistent with previous studies, although the magnitude of the correlation in broccoli is higher than in other foods evaluated in similar research. For example, Rumpf et al. reported a moderate correlation (R = 0.757) between ABTS and FC,[18] while Castañeda-Valbuena et al. found a lower correlation (R = 0.471) in extracts from mango by-products.[31] These results highlight broccoli as a particularly rich source of polyphenolic antioxidants. Furthermore, although polyphenols are the primary contributors, other non-phenolic compounds such as vitamins and carotenoids complement the antioxidant activity.[32] This synergistic combination could enhance broccoli's benefits in preventing oxidative stress-related diseases, such as cancer and cardiovascular diseases.

Moreover, different correlation coefficients have been reported, contrasting with studies where sample pretreatment is crucial for evaluating plant extracts. For instance, the choice of extraction solvent significantly impacts antioxidant capacity. Additionally, the polarity/solubility of the solvent can be a limiting factor when selecting the best method.[29,33] Differences in antioxidant activity are highlighted because each method is specific. For example, DPPH is suitable for hydrophobic compounds, while ABTS is appropriate for both hydrophilic and hydrophobic compounds.[18] The presence of hydroxycinnamic acids, a type of non-flavonoid phenolic compound, has been reported in Brassica species, showing greater affinity for hydrophilic solvents. Among these, p-coumaric, ferulic, and sinapic acids stand out for being hydrophilic compounds.[34]

CONCLUSION

The extracts of *Brassica oleracea var. italica* "broccoli" exhibit high phenolic content, particularly the hydroalcoholic extract, and demonstrate good antioxidant capacity, being more effective against the ABTS radical compared to the DPPH radical.

ACKNOWLEDGEMENT

The authors wish to thank Universidad César Vallejo for providing access to the laboratory of the Professional School of Nutrition and the research project with code P-2023-198, "Potencial fuente en polifenoles y antioxidantes de la especie *Myrcianthes discolor* (Kunt) McVaugh" for supplying the inputs and materials necessary for the study.

This research was approved by the Ethics Committee of the Professional School of Nutrition under the code CEI: PI-CEI-NUT-EST.2024-0005.

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

FUNDING

The article processing charge (APC) was funded by the Research Support Fund of Cesar Vallejo University.

ABBREVIATIONS

FC: Folin Ciocalteu; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid; Trolox: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; GAE: gallic acid equivalent; TE: Trolox equivalent; DM: dry matter.

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Cite this article: Florian VNE, Sisniegas CGM, Valdiviezo-Campos JE. Effect of Different Extraction Solvents on the Total Phenolic Content and Antioxidant Activity of Brassica *oleracea* var. *italica*. Pharmacogn J. 2025;17(1): 58-62.