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# Comparative Spectrophotometric Studies of Total Phenolics Content and Antioxidant Capacity Measured by DPPH and ABTS Methods in Green Vegetables

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## Abstract

Modern trends in nutrition imply the daily intake of fresh fruits and vegetables, with the statement that they are important sources of natural antioxidants that protect the body from oxidative stress and can contribute to the prevention of many chronic diseases. In these recommendations, green vegetables with high total phenolics content (TPC) are especially highlighted. However, one must ask whether the high total phenolics content of green vegetables, such as Brussels sprouts, cabbage, broccoli, parsley, borecole and spinach, is a reliable indicator of antioxidant activity for regulating free radicals in the body. The aim of this work is the spectrophotometric determination of TPC of hydromethanol extracts of selected green vegetables and its correlation with their antioxidant potential. For this purpose, DPPH and ABTS radical scavenging tests of extracts were performed. The obtained results indicate that Brussels sprouts and parsley exhibit the highest antioxidative activities, while cabbage extract shows lower values.

The Pearson correlation used to test the correlation between the TPC obtained for green vegetable extracts and the results of the DPPH and ABTS tests shows a moderate correlation between TPC and ABTS values, whereas the correlation for TPC and DPPH is statistically significant.

**Key words:** green vegetables, antioxidative activities, total phenol content (TPC), DPPH, ABTS

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## Introduction

Epidemiological studies overwhelmingly support the idea that nutrition is important in preventing many chronic diseases. A diet rich in natural antioxidants can significantly influence the increase of an organism's reactive antioxidant potential and reduce the risk of several diseases with a free radical origin.

A sufficient dietary antioxidant level stimulates immune functions and appropriately raises cellular defenses (1). Phenolic compounds, which are naturally occurring antioxidants, have been found to exhibit a variety of positive bioactivities, including anti-allergic, anti-inflammatory, and anti-mutagenic characteristics (2). Due to their safety and possible medicinal and nutritional benefits, natural antioxidants found in food and other plant material, such as bioactive phenolics, ascorbates, tocopherols and carotenoids, have drawn a lot of attention. Since plants are the primary source of natural antioxidants, goods produced from plants are the main supply of these substances for humans.

### Green Vegetables

For the purpose of this research, several green vegetables from different families were investigated, namely Brussels sprouts, cabbage, broccoli and borecole (*Brassicaceae*), spinach (*Amaranthaceae*) and parsley (*Apiaceae*). All of those have been regarded as significant components of the world's diet (3–5) and are known for their nutritive value and as rich sources of numerous bioactive substances with health-promoting properties (6). Since vegetables are good sources of various antioxidant compounds (phenolics, carotenoids, vitamin C), their consumption could be attributed as a natural kind of prevention and regulation of free radicals' level and oxidative stress (7).

### Oxidative Stress and Free Radicals

It is widely established that oxidative stress has a role in the etiology of diseases associated with a sedentary lifestyle, such as atherosclerosis, hypertension, diabetes mellitus, etc. According to that, oxidative stress is essentially an imbalance between the body's capacity to neutralize or detoxify free radicals through the action of antioxidants and the generation of free radicals (8).

An unpaired electron-containing molecule is referred to as a free radical which is a reactive species. They are formed by homolytic bond breaking in oxidation-reduction processes, thermal, photochemical, or high-energy radiation (5). They can act as oxidants or reductants by either giving or receiving an electron from other molecules. The hydroxyl radical ($OH^{\bullet}$), superoxide anion radical ($O^{2\bullet-}$), hydrogen peroxide ($H_2O_2$) and others are the oxygen-containing reactive species that are extremely reactive substances and can harm important components like DNA, proteins and lipids in cell membranes and the nucleus (9) and disrupt homeostasis (10). In addition to reactive oxygen species (ROS), reactive chlorine species (RCS) and reactive nitrogen species (RNS) were also described (11).

Phenols are aromatic chemicals with hydroxyl groups that are directly bonded to the benzene nucleus in place of the hydrogen atoms. Term “phenolic compounds” refers to a broad set of substances found in the plant kingdom and distinguished by having at least one aromatic ring with one or more hydroxyl groups attached. Since they have demonstrated promising antioxidant activity in both *in vivo* and *in vitro* studies, phenolic antioxidants are considered as ubiquitous plant secondary metabolites of great interest and tremendous posibilities for application (12–14). Nowadays, there is a trend toward the enhancement of antioxidant activity by the addition of plant materials rich in phenolic compounds to everyday consumption of food (15).

Antioxidants are chemicals that can neutralize the negative effects of the physiological oxidation process in living organisms by “neutralizing” free radicals to prevent the oxidation process (16). Free radicals take electrons from other molecules, causing harm to those molecules. Free radicals are countered by antioxidants by sacrificing some of their own electrons, respectively serving as a natural “off” switch for the free radicals. This aids in stopping a chain reaction that may have an impact on other cellular molecules and cells across the body. However, it’s crucial to understand that the term “antioxidant” refers to a chemical characteristic rather than a specific dietary characteristic. Together, all antioxidants form the antioxidant system, which is in charge of preventing the negative effects of free radicals (17).

Since there are many categories of antioxidants, for the purposes of this paper, the following divisions have been selected: endogenous (enzymes, proteins, and other metabolic products) and exogenous antioxidants (vitamins, flavonoids, phenolic acids, etc.); primary (flavonoids, vitamin E, phenolic compounds, etc.) and secondary antioxidants (citric acid, ascorbic acid, phospholipids, carotenoids, etc.) (18). Nevertheless, a number of studies showed that the best examples of antioxidants were those found in various fruits and vegetables because they belong to different categories of antioxidants and exhibit multiple mechanisms of antioxidant activity (19).

There are great structure varieties of plant phenolic compounds that possess antioxidant properties, e.g., flavonoids, phenolic acids, etc. Their activity depends largely on their chemical structure, particularly the number and position of hydroxyl groups (12). Every herbal drug rich in polyphenols contains specific active components that contribute to its antioxidant potential. For example, the fruit of chokeberry (*Aroniae fructus*) and the fruit of blueberry (*Myrtilli fructus*) are abundant in anthocyanins and tannins; in the rhizome of turmeric (*Curcumae rhizoma*) the dominant polyphenolic compounds are curcuminoids; the leaves of green tea (*Camelliae sinensis folium*) are rich in catechins, among which epigallocatechin gallate (EGCG) stands out; while coffee beans (*Coffeae semen*) are a significant source of chlorogenic acid. These substances not only prevent oxidative damage, but may also contribute to the prevention of chronic diseases caused by oxidative stress (12). Also, numerous research studies have revealed that gallic acid (3,4,5-trihydroxybenzoic acid, GA), a naturally occurring low molecular weight triphenolic molecule, has potent antioxidant properties among other polyphenols (20). It offers effective defense against oxidative harm from reactive species, such as hydroxyl

(HO•), superoxide ($O_2$•), and peroxyl (ROO•) radicals, which are frequently found in biological systems, as well as from non-radicals such hydrogen peroxide ($H_2O_2$) and hypochlorous acid (HOCl).

The aim of this study is comparative spectrophotometric determination of total phenolic content (TPC) in hydromethanol extracts of 6 green vegetables, as well as the determination of their antioxidative capacities, based on two assays, DPPH and ABTS. The results of the study are compared with already published literature data, and the relation between the obtained TPC, DPPH, and ABTS results are discussed.

## Experimental

### Materials and Methods

#### *Reagents*

ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), Trolox, DPPH (2,2-diphemyl-2-picrylhydrazyl), PBS buffer (phosphate buffered saline), $Na_2CO_3$, $K_2S_2O_8$, gallic acid, Folin-Ciocalteu (FC) reagent (Sigma-Aldrich), methanol (Merck), all p.a. purity grade, were used without further purification.

#### *Extract Preparation*

The selected fresh plant material was provided by the domestic producer of organically cultivated vegetables. In this way, under controlled growing conditions, the potential influence of pesticides and/or antibiotics on the tested parameters was avoided (21). Samples of fresh Brussels sprouts, cabbage, broccoli, borecole (*Brassicaceae*), spinach (*Amaranthaceae*), and parsley (*Apiaceae*) were used for this study. Each green vegetable was chopped, measured (5 g), placed in a 100 mL beaker, and extracted with 25 mL of 80% methanol. Extracts were sonicated in ultrasound bath for 30 min and then filtered (22).

#### *Instruments*

For all spectrophotometric measurements the *MAVRICA-17U* spectrophotometer (Colo Lab Experts) was used, using 1 cm of quartz cells. Measurements of pH were carried out using a Mettler Toledo mp 120 pH meter, equipped with a combination electrode.

### Determination Procedures

#### *Determination of Total Phenolic Content (TPC)*

The principle of this method is based on the reduction of metal ions present in the Folin–Ciocalteu reagent by phenolic compounds, resulting in a color change from yellow to a molybdenum-tungsten blue complex (often referred to as Prussian blue). The intensity of the blue color is directly proportional to the phenolic content in the sample and is typically measured spectrophotometrically at 765 nm.

The phosphomolybdic/phosphotungstic acid complexes are formed when phenolic chemicals transfer electrons to them under alkaline conditions (23). These electron transfers enable a color shift, which is then visible at 760 nm (24). The blue wavelength's absorbance can then be calibrated using a reference substance by applying the Beer-Lambert law, with GA employed as a standard, so the outcomes are expressed as gallic acid equivalents (25).

Determination of TPC was measured according to the modified Singleton method (26). 0.5 mL of prepared FC-water solution (1:10) was added in a 10 mL volumetric flask with 500 μL of extract, 1 mL of $Na_2CO_3$ (7.5 %) and filled to mark with deionized water, protected from light with aluminum foil. This process was repeated for each extract. As a control, a sample containing all reagents has also been made, but without adding any extract.

The resulting mixture was left for 30 min in the dark, at room temperature, after which the absorbance at 750 nm was measured.

The calibration curve of gallic acid (GA) is performed as the most frequently employed standard for TPC, and the presented results are expressed as gallic acid equivalents. The standard curve of GA obtained for TPC determination: $y = 0.0039x + 0.0101$, $R^2 = 0.9988$.

***DPPH Assay***

When the antioxidant reacts with the DPPH radical, it accepts an electron to transform into a stable magnetization molecule in the presence of hydrogen as an electron donor (27). As a result, the radical is reduced to DPPH, which results in discoloration of the solution (turns yellow) since the DPPH molecule absorbs light at shorter wavelengths than the DPPH radical.

To perform the DPPH test (28), 5 mL of previously prepared $DPPH^{\bullet}$ solution (0.004%) was transferred in a 10 mL volumetric flask, then 100 μL of the extract was added and filled up with methanol. The flask was covered with aluminum foil and the reaction mixture was stored in the dark for 1 hour. The absorbance of the solution ($A_{sample}$) was measured at a wavelength of 514 nm. The results were expressed as a percentage of DPPH inhibition according to the equation (1):

$$\%\ Inhibition\ of\ DPPH\ radical\ = \frac{A_{DPPH} - A_{sample}}{A_{DPPH}} \times 100 \qquad (1)$$

$A_{DPPH}$ is the absorbance of the DPPH control sample (5 mL of DPPH standard solution filed up to 10 mL with methanol) and $A_{sample}$ is the absorbance of samples after 30 min. To validate the procedure, Trolox solutions were utilized as standard compounds, and standard curve of Trolox was obtained for the DPPH assay in the concentration range 0.2–4.5 mg $L^{-1}$ ($y = 19.7845x - 0.2652$, $R^2 = 0.9988$).

### *ABTS Assay*

As a quick screening method, the green vegetable samples were tested for their capacity to scavenge ABTS free radicals ($ABTS^{\bullet+}$). With a few minor adjustments, the ABTS test was carried out as explained by Fang Hsu et al. (29).

The pre-generated dark green solution of the ABTS radical cation ($ABTS^{\bullet+}$) undergoes decolorization when interacting with an antioxidant to form the basis of the ABTS test. The ABTS solution fades, and then, as a result, the absorption spectrum is changed in the sense that the characteristic absorption lines from the VIS spectrum make it simple to quantitatively detect $ABTS^{\bullet+}$ scavenging (30). An easy way to identify an $ABTS^{\bullet+}$ formation is by the solution color shift from nearly colorless to deep bluish-green.

To measure the ABTS antiradical activity of the green vegetable samples, 100 µL of each extract was dissolved in 5 mL of $ABTS^{\bullet+}$ solution, mixed thoroughly, and then diluted up to 10 mL with PBS buffer. The volumetric flasks were covered with aluminum foil. The reaction mixtures were incubated at room temperature and their absorbance ($A_{sample}$) was measured at 734 nm after 3 min. The following equation (2) was used to express the results as a percentage of ABTS inhibition:

$$\%\ Inhibition\ of\ ABTS = \frac{A_{ABTS} - A_{sample}}{A_{ABTS}} \quad (2)$$

Where: $A_{ABTS}$ is the absorbance of the 5 mL of ABTS solution diluted up to 10 mL with PBS buffer. To validate the procedure, gallic acid solutions were used as standard compounds and in the concentration range of 0.1–1.8 mg $L^{-1}$ the calibration curve was obtained. The antioxidant activity against the ABTS radical was also expressed as the equivalent concentration of gallic acid corresponding to the respective percentage of inhibition.

### Statistics

Using the bivariate Pearson correlation, coefficients were calculated to estimate the linear correlation between the variables. All statistical calculations were performed using IBM SPSS software 26.0.

## Results and Discussion

### Results

When it is considered that the experiment was done exclusively with green vegetables, the color of obtained extracts is green but varies in its shades. As can be seen from Figure 1a, cabbage is the vegetable whose color differs the most from the others. The color obtained from cabbage ranges between light yellow and light green.

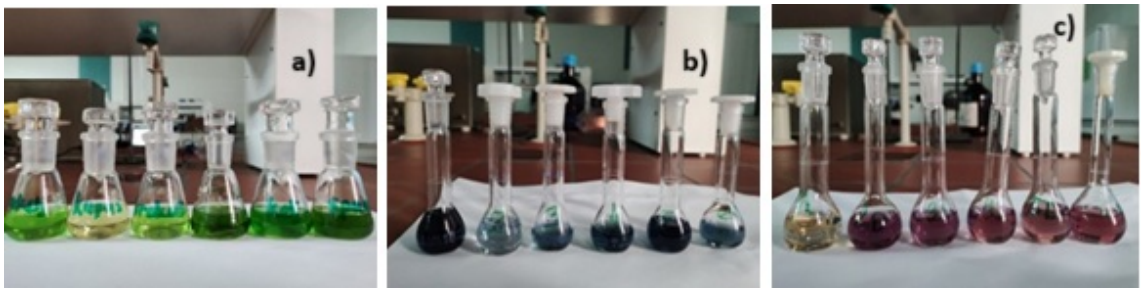


**Figure 1.** **a) Methanol/water extracts of green vegetables; b) Folin-Ciocalteu reagent with extracts; c) DPPH reagent with extracts (extracts displayed in the following order: Brussels sprouts, cabbage, broccoli, parsley, borecole and spinach are shown – viewed from left to right)**

**Slika 1.** **a) Metanol/vodeni ekstrakti zelenog povrća; b) Folin-Ciocalteu reagens sa ekstraktima; c) DPPH reagens sa ekstraktima (ekstrakti su prikazani po sledećem redosledu: prokelj, kupus, brokuli, peršun, kelj i spanać – gledano s leva na desno)**

Beyond their primary biological role in plant defense, phenolic compounds are well known for their antioxidant activity, and the assessment of total phenolic content (TPC) is a crucial step in evaluating the overall antioxidant capacity of natural samples. As shown in Figure 1b, all extracts developed a blue coloration after reacting with the Folin–Ciocalteu reagent, varying shades of intensity. In the FC assay, a darker blue color indicates a higher TPC, suggesting a greater concentration of phenolic compounds and, consequently, a potentially higher antioxidant capacity. The calculated TPC values for green vegetable extracts are presented in Table I. The gallic acid (GA) concentration used as a standard corresponds to an extract concentration of 50 mL $L^{-1}$.

The intensive discoloration of the DPPH solution (from purple to yellow) indicates strong anti-DPPH acitivity and was clearly noticeable for the extract of Brussels sprout (Figure 1c). Calculated values for antioxidant activity of green vegetables' extracts with DPPH and ABTS reagents are shown in Table I. Concentrations of Trolox and Gallic acid (GA) correspond to the extract concentration of 10 mL $L^{-1}$.

**Table I** Total phenolic content (TPC) and antioxidant activity of the tested green vegetable extracts

**Tabela I** Ukupan sadržaj fenola (TPC) i antoksidativna aktivnost ekstrakata testiranog zelenog povrća

| Test → | TPC | | DPPH | | | ABTS | | |
|---|---|---|---|---|---|---|---|---|
| Vegetable ↓ | $C_{GA}$, mg $L^{-1}$ | $m_{GA}/V_{ext.}$, mg $mL^{-1}$ | %INH | $C_{trolox}$, mg $L^{-1}$ | $m_{trolox}/V_{ext.}$, mg $mL^{-1}$ | %INH | $C_{GA}$, mg $L^{-1}$ | $m_{GA}/V_{ext.}$, mg $mL^{-1}$ |
| Brussels sprouts | 351.00 | 7.02 | 73.6 | 3.73 | 0.373 | 76.0 | 1.38 | 0.138 |
| Cabbage | 29.19 | 0.58 | 2.1 | 0.12 | 0.012 | 20.2 | 0.29 | 0.029 |
| Broccoli | 103.80 | 2.08 | 10.0 | 0.52 | 0.052 | 29.4 | 0.88 | 0.008 |
| Parsley | 192.78 | 3.85 | 10.2 | 0.53 | 0.053 | 52.7 | 0.93 | 0.093 |
| Borecole | 239.70 | 4.79 | 30.1 | 1.53 | 0.153 | 36.3 | 0.61 | 0.061 |
| Spinach | 128.67 | 2.57 | 0.2 | 0.02 | 0.002 | 64.5 | 1.15 | 0.115 |

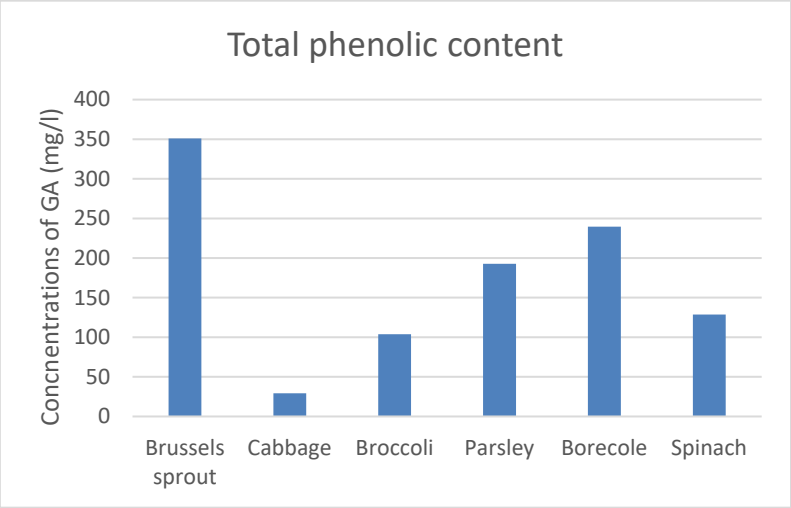


**Figure 2. Total phenolics content in green vegetables**

**Slika 2. Sadržaj ukupnih fenola u zelenom povrću**

The obtained results indicate significant differences in the total phenolic content (TPC) among the analyzed methanolic green vegetable extracts. Based on the results presented in Table I and Figure 2., the highest TPC value was recorded in Brussels sprouts, with 351.00 mg $L^{-1}$ GA, confirming its status as an exceptionally rich source of antioxidants. High TPC levels were also observed in borecole (239.70 mg $L^{-1}$ GA) and parsley (192.78 mg $L^{-1}$ GA), while broccoli (103.80 mg $L^{-1}$ GA) and spinach (128.67 mg $L^{-1}$ GA) showed moderate values. The lowest phenolic content was found in cabbage, with only 29.19 mg $L^{-1}$ GA, which is consistent with previously published results (31).

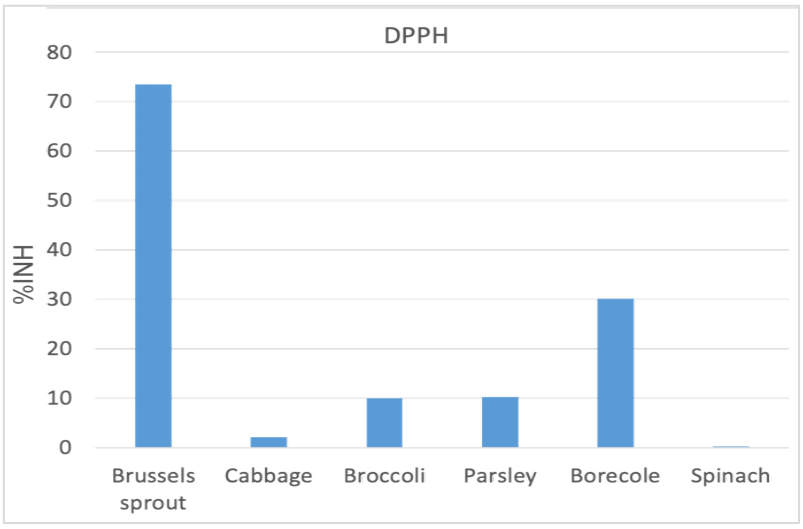


**Figure 3.** **The antioxidant activity of methanolic extracts of the green vegetable samples determined using the DPPH method**

**Slika 3.** **Antioksidativna aktivnost metanolnih ekstrakata uzoraka zelenog povrća određena DPPH metodom**

From the obtained results of the DPPH assay, it can be observed that Brussels sprouts (73.6 %INH), followed by the Borecole (30.1 %INH) sample, show significantly higher antioxidant activity compared to the other analyzed samples. According to other studies (31), Brussels sprouts are known to be a very good source of antioxidants, which was established in this experiment as well. Other values were much lower, with the lowest values measured for cabbage (2.1 %INH) and spinach (0.2 %INH).

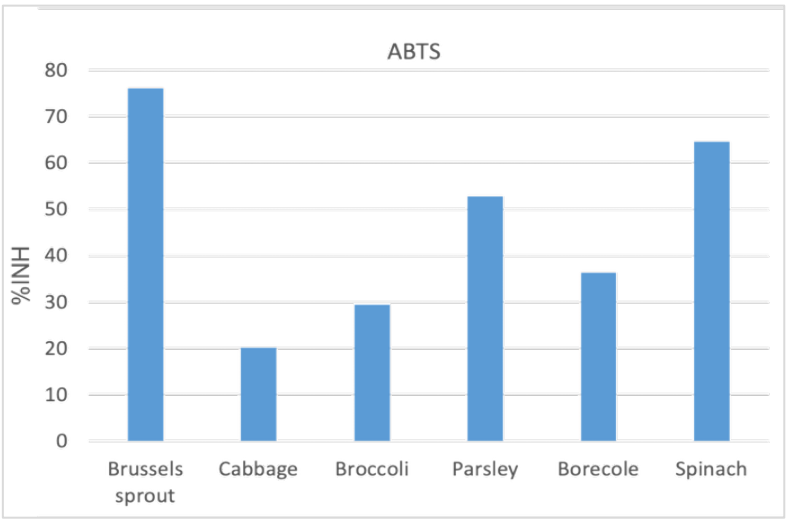


**Figure 4.** **The antioxidant activity of methanolic extracts of the green vegetable samples determined using the ABTS method**

**Slika 4.** **Antioksidativna aktivnost metanolnih ekstrakata uzoraka zelenog povrća određena ABTS metodom**

As seen in Figure 4. and Table I, samples of the Brussels sprouts (76.0 %INH), spinach (64.5 %INH) and parsley (52.7 %INH) showed greater activity to scavenge ABTS radical compared to the others analyzed samples. As expected, the smallest inhibition of the ABTS radical was exhibited by cabbage (20.2 %INH).

## Discussion

In this study, DPPH and ABTS assays were used, as they are among the most commonly applied and well-standardized methods for the screening of antioxidant activity. Their combined application is particularly justified in the analysis of complex plant extracts, as they provide information on the ability of molecules to neutralize synthetic free radicals in different media (31, 32). The DPPH assay is performed in an organic solvent and primarily detects lipophilic antioxidants, whereas the ABTS assay can be applied in both aqueous and organic media, allowing for the detection of a broader spectrum of compounds, including hydrophilic ones.

Brussels sprouts can be considered the winner in this study. Its extract exhibited the highest total phenolic content values and according to both the DPPH and ABTS methods, Brussels sprouts also demonstrated very strong antioxidant activity. These findings are consistent with previous studies (33–35).

However, there are some minor differences among the results of these three methods. This could be the consequence of different phenolics composition of the investigated extracts. The presence of some compounds, other than phenolics, which also exhibit antioxidant activity, should also be considered. For example, plants belonging to *Brassicaceae*, besides polyphenols, such as flavonoids and phenolic acids, are rich in glucosinolates, carotenoids and vitamin C, all known for their antioxidant and health promoting properties (36, 37).

The high TPC in Brussels sprouts can be attributed to their rich content of flavonoids, phenolic acids, and glucosinolates, compounds with well-documented antioxidant properties (34). Similarly, Borecole and parsley are known for their high levels of phenolic compounds, contributing to their nutritional value and their role in preventing oxidative stress (38).

The lower phenolic content observed in cabbage compared to other analyzed samples may be due to genetic differences, degree of maturity, and the distribution of phenolic compounds within plant tissues. This finding is supported by Li et al. (33), who reported significantly lower TPC in white cabbage extract compared to other *Brassica* species. White cabbage contains fewer polyphenols because, both genetically and physiologically, it is not primed to produce high levels of these protective compounds (33, 38).

Interestingly, although broccoli is often regarded as a “superfood”, in this study it exhibited only moderate TPC levels. However, it is important to note that the total antioxidant capacity of a plant does not depend solely on phenolic content, but also on the presence of other antioxidant components such as vitamins C and E, carotenoids, and

sulforaphane, which are particularly abundant in broccoli (39). The Folin–Ciocalteu assay for the total phenol determination interferes with many other naturally occurring compounds, and many efforts were made to improve the selectivity of this determination, including the pre-extraction procedures (40, 41). However, the method is still helpful for screening purposes, giving insight into the antioxidative capacities of the tested samples.

The results of antioxidant activity tested by DPPH and ABTS assays revealed significant variation in free radical scavenging capacity depending on the assay applied. In the ABTS assay, the highest inhibition was observed in Brussels sprouts (76.0%), spinach (64.5%), and parsley (52.7%). In the DPPH assay, Brussels sprouts again showed the strongest activity (73.6%), followed by borecole (30.1%), while parsley and broccoli showed similarly low values (10.1% and 10.0%, respectively). Notably, spinach demonstrated high antioxidant capacity in the ABTS assay, but almost no activity in the DPPH assay (0.2%), indicating a strong assay-specific response. The lowest inhibition values in both assays were recorded for cabbage (20.2% ABTS; 2.1% DPPH).

The correlation between the TPC obtained for green vegetable extracts and the results of the DPPH and ABTS tests was statistically analyzed using the Pearson correlation. Although there are some similarities in the profile of the obtained results, as indicated by the correlation coefficient of 0.663, the correlation between TPC and ABTS values is insignificant. On the contrary, the correlation coefficient of 0.899 for TPC and DPPH is statistically significant at the 0.05 level.

Although the antioxidant activity did not show a perfect correlation with the total phenolic content (TPC), extracts of Brussels sprouts, borecole, and parsley, which had the highest TPC values, also exhibited the strongest antioxidant activity in both assays. This suggests that phenolic compounds play an important but not exclusive role in the overall antioxidant potential of plant extracts. Certain discrepancies were observed that cannot be explained solely by TPC values. For instance, spinach showed a higher antioxidant capacity compared to broccoli, despite broccoli having a higher TPC. This suggests the presence of other antioxidant-active compounds in spinach, such as vitamin C, carotenoids, and glutathione (34).

The results obtained in this study are in agreement with a systematic review conducted by Halvorsen et al., which showed that parsley, spinach, and kale are among the plant species with the highest total antioxidant capacity. In contrast, broccoli and white cabbage showed moderate values (42).

When comparing the DPPH and ABTS assays, the results were not entirely consistent, and the inhibition percentage was always higher in the ABTS assay. This difference may be attributed to the distinct mechanisms and sensitivities of the two methods. The ABTS assay is capable of reacting with a broader range of antioxidant compounds, including both hydrophilic and lipophilic substances, whereas the DPPH assay primarily reacts with lipophilic antioxidants (43–45). Among the tested samples, Brussels sprouts showed the highest radical inhibition in both tests, and in general, plants from the *Brassicaceae* family (except cabbage) exhibited a higher percentage of DPPH

inhibition compared to spinach, parsley, and cabbage. This may be explained by their rich content of antioxidants such as carotenoids and vitamin E, which are more reactive with the DPPH radical due to its solubility in organic solvents. It is preferable to use both methods rather than relying on just one to ensure greater reliability in antioxidant activity determination. However, differences between the methods can also be influenced by experimental conditions, such as pH, temperature, and solvent polarity. For example, DPPH was prepared in methanol, whereas ABTS was prepared in water, which may affect the reactivity of antioxidant compounds present in investigated extracts (38).

To obtain a more comprehensive assessment of the antioxidant potential of the tested samples, it is helpful to apply other relevant methods as well, such as the CUPRAC assay and electrochemical techniques based on the elimination of the superoxide radical ($O^{2-}\cdot$) (44, 46). The CUPRAC assay, which is planned for future research, is of particular importance because it is conducted at physiological pH (~7) and measures the overall reducing capacity, making it more suitable for evaluating antioxidant activity under conditions closer to those in biological systems.

## Conclusion

The obtained results indicate that the high total phenolic content does influence the antioxidant activity of green vegetables from the *Brassicaceae, Amaranthaceae*, and *Apiaceae* family, like Brussels sprouts, cabbage, broccoli, borecole, spinach and parsley. The results suggest that Brussels sprouts and parsley may serve as highly valuable natural sources of antioxidants in the human diet. At the same time, the lower values observed for cabbage extract highlight the importance of combining different plant isolates in order to achieve optimal intake of protective phytochemicals. Of course, the next step in continuing this research is to consider replacing methanol with some adequate harmless, preferably “green” and eco-friendly solvent, maintaining antioxidant activity. Additionally, the influence of thermal processing and freezing on antioxidant activity could be assessed.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Author contributions

**S.D.**: Investigation, Writing – original draft preparation; **S.U.M**.: Data Interpretation, Writing – review and editing; **J.K.M**.: Writing – review and editing; **L.P**.: Formal analysis, Validation; **A.J.L**: Conceptualization, Writing – review and editing, Supervision.

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# Uporedno spektrofotometrijsko ispitivanje ukupnog sadržaja fenola i antioksidativnog kapaciteta merenih DPPH i ABTS metodama u zelenom povrću

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## Kratak sadržaj

Savremeni trendovi u ishrani podrazumevaju svakodnevni unos svežeg voća i povrća, uz konstataciju da su oni važni izvori prirodnih antioksidanata koji štite organizam od oksidativnog stresa i mogu doprineti prevenciji mnogih hroničnih bolesti. U ovim preporukama posebno se ističe zeleno povrće sa visokim ukupnim sadržajem fenola (TPC). Međutim, mora se postaviti pitanje da li je visok ukupan sadržaj fenola u zelenom povrću, kao što su prokelj, kupus, brokoli, peršun, kelj i spanać, pouzdan pokazatelj antioksidativne aktivnosti za regulisanje slobodnih radikala u organizmu. Cilj ovog rada je spektrofotometrijsko određivanje TPC i utvrđivanje korelacije između TPC i antioksidativnog potencijala metanol-vodenih ekstrakata odabranog zelenog povrća. U tu svrhu sa ekstraktima povrća urađeni su DPPH i ABTS testovi uklanjanja radikala. Dobijeni rezultati ukazuju na to da prokelj i peršun pokazuju najveću antioksidativnu aktivnost, dok ekstrakt kupusa pokazuje niže vrednosti. Pirsonova korelacija koja je korišćena za testiranje korelacije između TPC dobijene za ekstrakte zelenog povrća i rezultata DPPH i ABTS testova pokazuje umerenu korelaciju između vrednosti TPC i ABTS, dok je korelacija za TPC i DPPH statistički značajna.

**Ključne reči:** zeleno povrće, antioksidativna aktivnost, ukupan sadržaj fenola (TPC), DPPH, ABTS