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DPPH radical-scavenging activity of the Brkulja wheatgrass juice and its bioactive compound correlation

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Abstract

The antioxidant potential of wheatgrass juice derived from the ancient Brkulja wheat variety was investigated to assess its functional and nutraceutical value. As a heritage landrace, the Brkulja wheat showed genetic resilience and great antioxidant and nutritional values making it a promising source of bioactive compounds. Wheatgrass was harvested at the early jointing stage, and the extracted juice was analysed for total phenolic content, flavonoid concentration, and antioxidant activity using DPPH radical scavenging assay. Results demonstrated that the Brkulja wheatgrass juice possesses strong antioxidant capacity with an IC_{50} value of 0.75 µg/mL. The DPPH inhibition percentages ranged from 88.83% at the highest concentration to 23.75% at the lowest concentration, showing direct relationship between juice concentration and inhibition percentage. The total phenolic content ranged from 25.8 to 42.5 mg GAE/g, while flavonoid concentration ranged from 9.7 to 18.3 mg QE/g, both showing strong positive correlations with DPPH inhibition ($r > 0.9$). These findings support the use of the Brkulja wheatgrass juice as a natural antioxidant source and emphasize the importance of preserving ancient wheat varieties for health-promoting applications.

Key words: Antioxidant, Juice wheatgrass, Polyphenols, Health benefit.

Introduction

Ancient wheat varieties have gained significant attention in recent years due to their potential health benefits and superior nutritional profiles compared to modern wheat cultivars. These heritage varieties, which have remained largely unchanged over the last hundred years, are increasingly recognized for their enhanced bioactive compound content and functional properties (Dinu et al., 2018). Among these ancient varieties, the Brkulja wheat (*Triticum aestivum* L.) (Peña-Bautista et al., 2017) stands as one of the oldest original varieties grown in the Kneževo region of Bosnia and Herzegovina, tracing its roots back to the 1960s. During the era of hybridization, where new hybrid varieties surged in popularity, Brkulja faced a decline, surviving only in extensive agricultural areas like the mountainous terrains of the Kneževo/Milan Knezina region.

Wheatgrass, the young grass of the wheat plant, has been used as a healthy food for over 80 years. It is particularly rich in chlorophyll, vitamins, minerals, and various bioactive compounds with potential health benefits. Wheatgrass juice is consumed as a functional food and dietary supplement due to its purported antioxidant, anti-inflammatory, and detoxifying properties. The consumption of wheatgrass juice has been associated with various health benefits, including improved immune function, reduced oxidative stress, and protection against chronic diseases (Priyanka et al., 2024). Oxidative stress, characterized by an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defence mechanisms, plays a crucial role in the pathogenesis of numerous chronic diseases, including cardiovascular diseases, cancer, diabetes, and neurodegenerative disorders (Pizzino et al., 2017). Antioxidants are compounds that can neutralize free radicals and prevent oxidative damage to cellular components. Plant-derived antioxidants have gained considerable attention due to their potential role in disease prevention and health promotion.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay is widely used to evaluate the antioxidant capacity of plant extracts and natural products. It is a stable free radical that appears purple in solution and becomes colourless or pale yellow when neutralized by antioxidants. The degree of discoloration indicates the scavenging potential of the antioxidant compounds, which is quantified by measuring the decrease in absorbance at 517 nm (Gulcin & Alwasel, 2023).

Despite the growing interest in ancient wheat varieties and wheatgrass juice, there is limited scientific evidence regarding the antioxidant properties of wheatgrass derived from specific ancient wheat varieties, particularly the Brkulja wheat traditionally associated with the Kneževo region. This study contributes to the existing research by evaluating the antioxidant potential of wheatgrass juice from the ancient Brkulja wheat variety using the DPPH radical scavenging assay, as well as determining its total phenolic content and flavonoid concentration. The findings of this study will contribute to our understanding of the functional properties of the Brkulja wheatgrass and its potential applications in health promotion and disease prevention.

Material and Methods

Wheatgrass cultivation and juice extraction

Wheatgrass from the ancient Brkulja wheat variety was grown under controlled conditions (Figure 1).

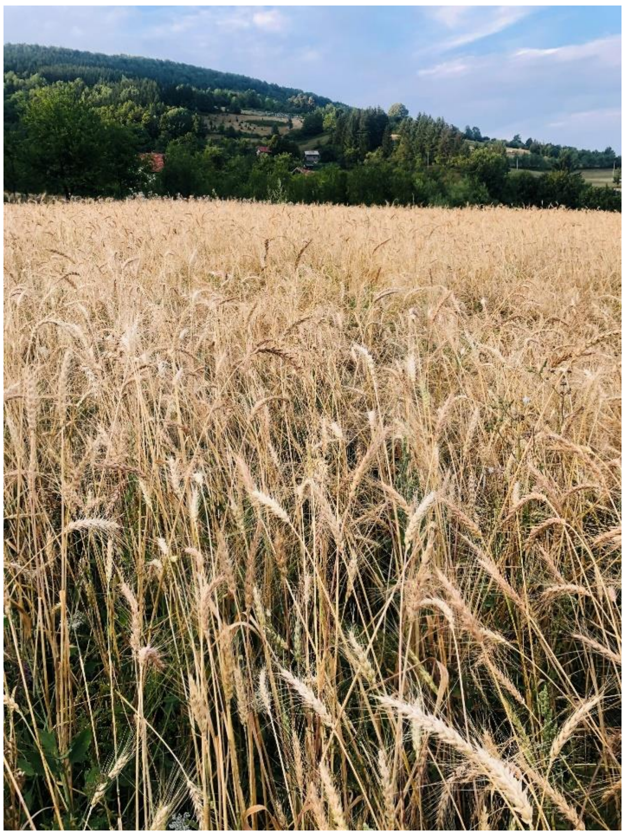

Fig. 1 - A field with the ancient Brkulja wheat in Kneževo, Republic of Srpska

Seeds provided by the seed variety owner (Familyholding Bajić, Kneževo, Republic of Srpska) were soaked in water for 12 hours prior to planting in organic soil. The plants were grown under natural light conditions at room temperature (22-25°C) and watered daily. Wheatgrass was harvested at the early jointing stage, approximately 10-14 days after germination, when it reached a height of 15-20 cm. This stage was selected as previous studies have indicated that the antioxidant activity of wheatgrass is optimal at this growth phase. Fresh wheatgrass was thoroughly washed with distilled water to remove any soil particles and contaminants. The clean wheatgrass was then processed using a cold-press juicer (KUVINGS REVO830, Italy) to extract the juice while preserving heat-sensitive bioactive compounds. The extracted juice was immediately filtered through a fine mesh to remove any solid particles and used for subsequent analysis without any further processing to maintain its natural composition and bioactivity.

DPPH radical neutralization assay

The antioxidant activity of the Brkulja wheatgrass juice was assessed using the DPPH radical scavenging method with minor modifications from previously reported protocols. Briefly, different volumes of juice (10–200 μL) were mixed with 0.5 mM DPPH solution in methanol to a final volume of 2 mL and incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm using a UV–VIS spectrophotometer.

The percentage of radical inhibition was calculated as:

$$I\ (\%) = [(Ak - Aa)/Ak] \times 100,$$

where

Ak is the absorbance of the control, and

Aa is the absorbance of the sample.

IC_{50} values were obtained by plotting inhibition (%) against sample concentration and fitting a dose–response curve using a four-parameter logistic model.

Determination of total phenolic content

The total phenolic content (TPC) of the Brkulja wheatgrass juice was determined using the Folin-Ciocalteu method (Nikolaeva et al., 2022). Briefly, different volumes of the wheatgrass juice (10, 25, 50, 100, and 200 μL) were mixed with 0.5 mL of Folin-Ciocalteu reagent (diluted 1:10 with distilled water) and 1.5 mL of 7.5% sodium carbonate solution. The mixtures were incubated at room temperature for 30 minutes in the dark, and the absorbance was measured at 765 nm using a UV-visible spectrophotometer. Gallic acid was used as a standard, and the results were expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g). A calibration curve was prepared using gallic acid solutions at concentrations ranging from 10 to 100 µg/mL.

Determination of flavonoid concentration

The flavonoid concentration of the Brkulja wheatgrass juice was determined using the aluminum chloride colorimetric method (Shraim et al., 2021). Different volumes of wheatgrass juice (10, 25, 50, 100, and 200 µL) were mixed with 0.3 mL of 5% sodium nitrite solution. After 5 minutes, 0.3 mL of 10% aluminium chloride solution was added, followed by 2 mL of 1 M sodium hydroxide after another 5 minutes. The volume was adjusted to 10 mL with distilled water, and the absorbance was measured at 510 nm using a UV-visible spectrophotometer. Quercetin was used as a standard, and the results were expressed as milligrams of quercetin equivalents per gram of the dry weigt (mg QE/g). A calibration curve was prepared using quercetin solutions at concentrations ranging from 10 to 100 µg/mL.

Results and Discussion

DPPH Radical Scavenging Activity

The antioxidant activity of the Brkulja wheatgrass juice was evaluated using the DPPH radical scavenging assay. The results of the DPPH inhibition percentages at different volumes of wheatgrass juice are presented in Table 1. The absorbance values were measured at 517 nm after 30 minutes of incubation with the DPPH reagent.

Tab. 1 - DPPH radical scavenging activity of the Brkulja wheatgrass juice at different concentrations

Volume (µL)	Concentration (µg/mL)	Inhibition (%)
200	10	88.83
100	5	84.52
50	2.5	82.48
25	1.25	65.27
10	0.5	23.75

The results demonstrated a clear concentration-dependent increase in the DPPH radical scavenging activity of the Brkulja wheatgrass juice, highlighting a pattern commonly observed in plant-based extracts. Several factors could contribute to this concentration-dependent increase in antioxidant activity. At higher concentrations, the increased presence of DPPH-scavenging compounds, along with the potential for certain components in wheatgrass juice to exhibit greater efficacy or synergistic interactions, may collectively enhance the overall free radical scavenging capacity

Dose-Response Relationship and IC_{50} Determination

To further characterize the antioxidant capacity of the Brkulja wheatgrass juice, a dose-response curve was generated by plotting the DPPH inhibition percentages against the estimated concentration of the juice (Figure 2). The concentration was calculated assuming a linear relationship between volume and concentration, with 1 µL of juice corresponding to approximately 0.05 µg of extract.

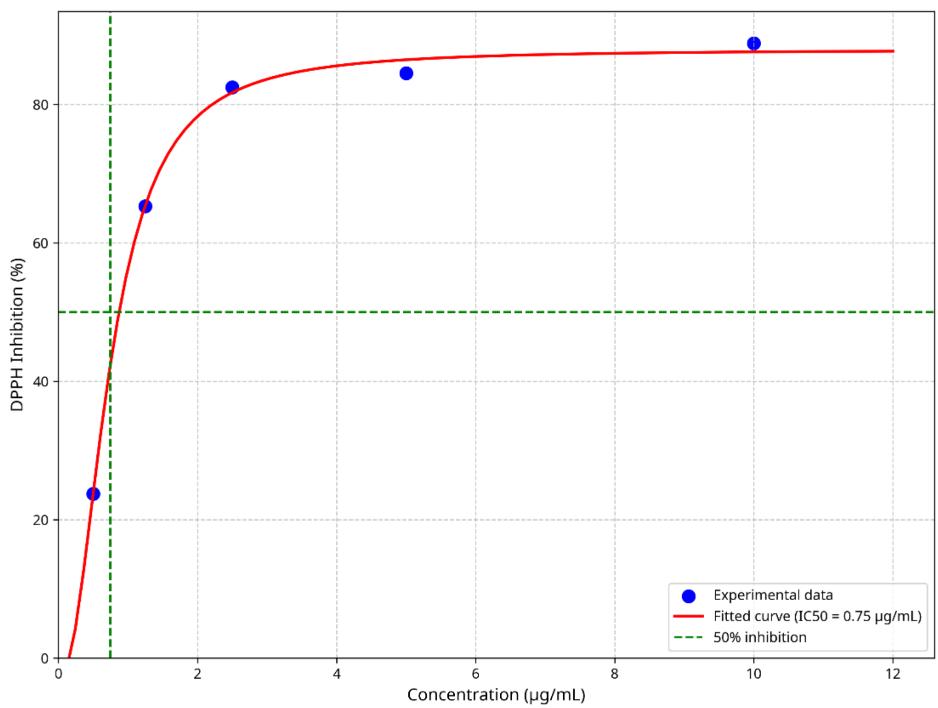

Fig. 1 - DPPH activity of the Brkulja wheatgrass juice

The dose-response curve exhibited a non-linear relationship, which is typical for antioxidant compounds. The curve was fitted using a four-parameter logistic function to determine the IC_{50} value, which represents the concentration required to inhibit 50% of DPPH radicals. The IC_{50} value for the Brkulja wheatgrass juice was calculated to be 0.75 μg/mL. This value indicates a strong antioxidant capacity, as lower IC_{50} values correspond to higher antioxidant activity. The calculated IC_{50} value is comparable to or better than those reported for other natural antioxidants in the literature, suggesting that the Brkulja wheatgrass juice possesses potent free radical scavenging properties (Itam et al., 2021). The dose-response curve also revealed that the antioxidant activity of the Brkulja wheatgrass juice is concentration-dependent, with a steep increase in inhibition percentage observed at higher concentrations. This pattern suggests that the optimal antioxidant activity is achieved at relatively low concentrations, which has important implications for the practical applications of the Brkulja wheatgrass juice as a natural antioxidant source.

Total phenolic content and flavonoid concentration

In addition to DPPH radical scavenging activity, the total phenolic content (TPC) and flavonoid concentration of the Brkulja wheatgrass juice were determined at different volumes. The results are presented in Table 2.

Tab. 2 - The total phenolic content, flavonoid concentration, and DPPH inhibition of the Brkulja wheatgrass juice at different volumes

Volume (μL)	TPC (mg GAE/g)	Flavonoids (mg QE/g)	DPPH Inhibition (%)
200	42.5	18.3	88.83
100	38.2	16.5	84.52
50	35.7	14.9	82.48
25	30.1	12.2	65.27
10	25.8	9.7	23.75

The total phenolic content ranged from 25.8 mg GAE/g at the lowest volume (10 μL) to 42.5 mg GAE/g at the highest volume (200 μL). Similarly, the flavonoid concentration ranged from 9.7 mg QE/g at 10 μL to 18.3 mg QE/g at 200 μL. These results indicate that both TPC and flavonoid concentration follow a similar pattern to DPPH inhibition, with lower values observed at lower volumes of wheatgrass juice.

Relationship between volume and bioactive compounds

The relationship between the volume of the Brkulja wheatgrass juice and its bioactive compound content is illustrated in Figure 3A and 3B. Both TPC and flavonoid concentration showed an increasing trend with increasing volume, which parallels the pattern observed for DPPH inhibition.

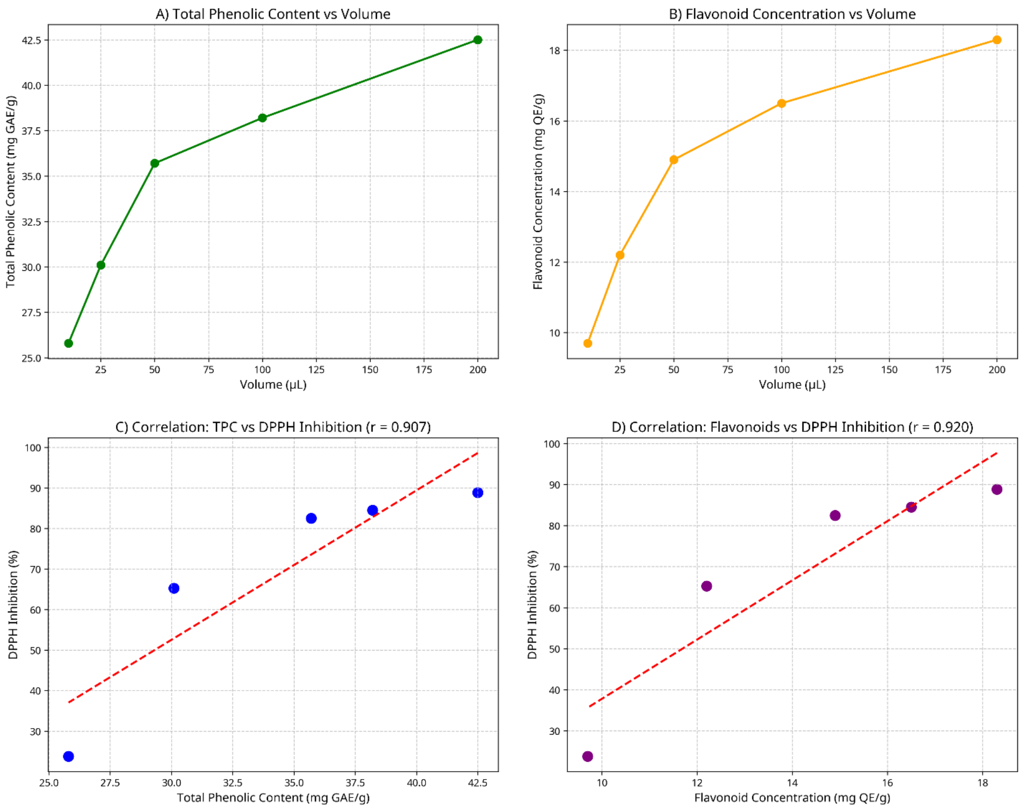

Fig. 2 - Relationship between bioactive compound content and antioxidant activity of the Brkulja wheatgrass juice

Correlation between bioactive compounds and antioxidant activity

To better understand the relationship between the bioactive compounds and antioxidant activity, correlation analyses were performed. The results revealed strong positive correlations between TPC and DPPH inhibition (r = 0.907), flavonoids and DPPH inhibition (r = 0.920), and TPC and flavonoids (r = 0.999), as shown in Figure 3C and 3D.

The strong correlation between TPC and DPPH inhibition suggests that phenolic compounds are major contributors to the antioxidant activity of the Brkulja wheatgrass juice. Similarly, the high correlation between flavonoid concentration and DPPH inhibition indicates that flavonoids play a significant role in the free radical scavenging capacity of the juice.

The near-perfect correlation between TPC and flavonoid concentration (r = 0.999) suggests that these bioactive compounds are closely related in the

Brkulja wheatgrass juice. This is consistent with the fact that flavonoids are a subclass of phenolic compounds, and their levels often change proportionally in plant extracts.

Figure 4 presents a correlation heatmap that visually represents the relationships between TPC, flavonoid concentration, and DPPH inhibition.

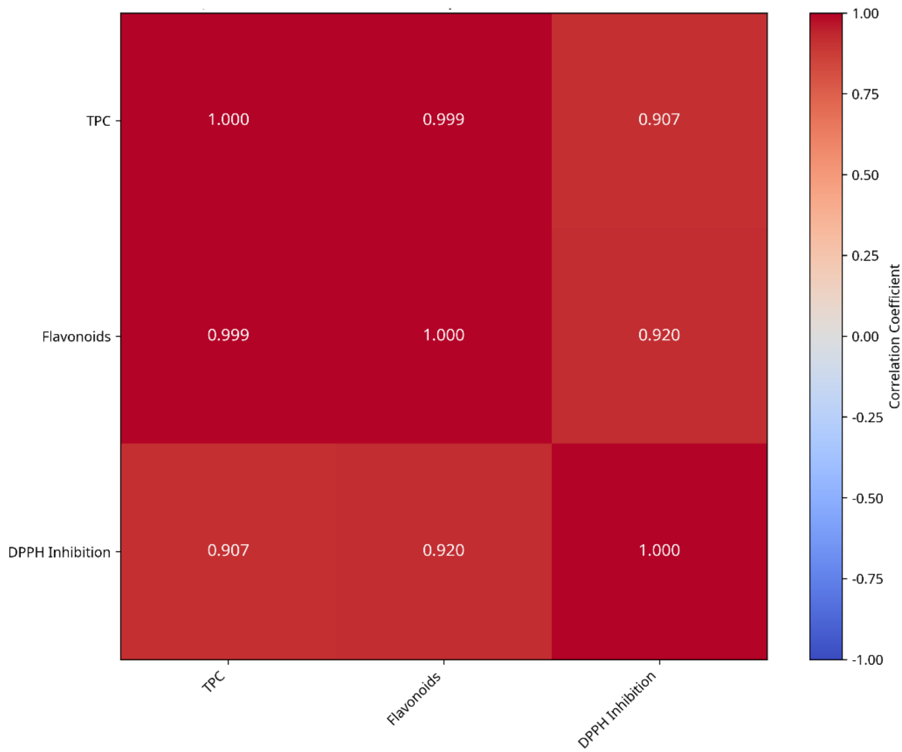

Fig. 3 - Correlation heatmap of antioxidant parameters

These findings highlight the importance of phenolic compounds and flavonoids in the antioxidant activity of the Brkulja wheatgrass juice and suggest that these bioactive compounds could be used as indicators of the juice's antioxidant potential. The results also support the traditional use of wheatgrass juice as a source of natural antioxidants and emphasize the value of ancient wheat varieties like Brkulja in providing health-promoting bioactive compounds. Supporting the production and sale of the Brkulja juice creates income-generating opportunities for rural farmers, contributing to local economic development.

Interpretation of DPPH Results

The DPPH radical scavenging assay results revealed a significant antioxidant capacity of the Brkulja wheatgrass juice, with an IC_{50} value of 0.75 μg/mL. Under the same experimental conditions, ascorbic acid showed slightly higher activity, with an IC_{50} of 0.6 μg/mL This value indicates that a relatively low concentration of the Brkulja wheatgrass juice is required to neutralize 50% of DPPH radicals, suggesting potent antioxidant properties. The observed directional relationship between juice volume and DPPH inhibition percentage represents a notable trend that merits further investigation. Several factors could contribute to this concentration-dependent effect. First, it is possible that at higher concentrations, certain compounds in the wheatgrass juice become more active or interact synergistically, enhancing their ability to scavenge free radicals. This phenomenon is commonly observed in plant extracts, where antioxidant activity increases with concentration up to a certain threshold. Second, the colour intensity of the wheatgrass juice at higher volumes might influence the spectrophotometric measurements; however, in this case, the observed trend is consistent with increased antioxidant activity. Third, the complex mixture of bioactive compounds in wheatgrass juice may exhibit enhanced kinetics of reaction with DPPH radicals at higher concentrations.

Comparison with other wheat varieties

The antioxidant capacity of wheatgrass juice from different wheat varieties has been reported in the literature, allowing for comparative analysis. According to Parit et al. (2018), the percentage of radical scavenging activity of wheatgrass extract by DPPH was 15%, 22%, and 30% after 0, 8, and 16 days after germination, respectively. In our experiments, the Brkulja wheatgrass juice showed inhibition percentages in the range of 23.75–88.83%, depending on the concentration. These findings should be interpreted with caution, as the DPPH assay is a screening method with well-known limitations and results may vary between laboratories. Nevertheless, our observations are in line with the study of Dapčević-Hadnađev et al. (2022), who reported that ancient wheat varieties (emmer, spelt, and khorasan) tended to release higher levels of bound phenolics and exhibited greater solubility compared to modern wheat varieties. The superior antioxidant capacity of the Brkulja wheatgrass juice compared to other wheat varieties previously studied could be attributed to its unique genetic makeup and the preservation of beneficial traits that might have been lost or reduced in modern wheat cultivars due to intensive breeding programs. Ancient wheat varieties like Brkulja have been less subjected to selective breeding for yield and technological properties, potentially retaining higher levels of bioactive compounds with antioxidant properties.

Factors affecting antioxidant activity in wheatgrass

Several factors can influence the antioxidant activity of wheatgrass juice, including the wheat variety, growth conditions, harvesting time, and the extraction method. The growth stage of wheatgrass is particularly important, as the concentration of bioactive compounds changes during plant development. According to Parit et al. (2018), the antioxidant activity of wheatgrass increases with the age of seedlings, with the highest activity observed at 16 days after germination. In our study, the Brkulja wheatgrass was harvested at the early jointing stage (10-14 days after germination), which is generally considered optimal for juice extraction. At this stage, wheatgrass contains high levels of chlorophyll, vitamins, minerals, and various bioactive compounds with antioxidant properties. The cold-press juicing method applied in our study may have contributed to the preservation of heat-sensitive bioactive compounds, which could partly explain the antioxidant activity observed. However, this remains a hypothesis, as no comparison with other extraction methods was performed. It should also be noted that environmental factors such as soil composition, water quality, light intensity, and temperature are known to influence the synthesis and accumulation of bioactive compounds in wheatgrass. In our study, controlled growing conditions were used in an attempt to reduce such variations and provide a more standardized environment.

Relationship between antioxidant capacity and bioactive compounds

The antioxidant capacity of wheatgrass juice is largely attributed to its rich content of bioactive compounds, including phenolic acids, flavonoids, chlorophyll, and various enzymes. Phenolic compounds, in particular, are known for their strong antioxidant properties due to their ability to donate hydrogen atoms or electrons to free radicals, thereby neutralizing them. According to Dinu et al. (2018), ancient wheat varieties generally contain higher levels of phenolic compounds compared to modern wheat cultivars. These phenolic compounds, including ferulic acid, p-coumaric acid, and vanillic acid, contribute significantly to the antioxidant capacity of wheat and wheat-derived products. The antioxidant activity of the Brkulja wheatgrass juice may be partly explained by direct free radical scavenging, as indicated by the DPPH assay, while additional mechanisms such as metal chelation or modulation of antioxidant defences cannot be excluded. These possible modes of action remain hypothetical and require further confirmation. Future studies would provide a more comprehensive understanding of the mechanisms underlying the observed antioxidant activity and could guide the development of optimized cultivation and extraction methods to maximize the health-promoting properties of the Brkulja wheatgrass juice.

Relationship between phenolic content, flavonoid concentration, and antioxidant activity

In our study, the Brkulja wheatgrass juice showed high correlations between TPC and DPPH inhibition (r = 0.907) and between flavonoids and DPPH inhibition (r = 0.920), which is expected since such associations are commonly observed in antioxidant assays (Parit et al., 2018). Moreover, Dinu et al. (2018) found that ancient wheat varieties generally contained higher levels of phenolic compounds compared to modern wheat cultivars, which contributed to their enhanced antioxidant properties. These findings, consistent with earlier reports on wheatgrass and ancient wheat varieties, suggest that phenolic compounds, including flavonoids, may contribute to the observed antioxidant activity, although the exact mechanisms remain to be clarified.

Conclusion

The strong antioxidant activity observed in the Brkulja wheatgrass juice can be attributed to its rich content of bioactive compounds, including phenolic acids, flavonoids, chlorophyll, and various enzymes. As an ancient wheat variety, the Brkulja wheat has likely retained beneficial traits that might have been reduced in modern wheat cultivars due to intensive breeding programs focused primarily on yield and technological properties. The findings of this study have important implications for the potential applications of the Brkulja wheatgrass juice as a natural antioxidant source. The preservation and cultivation of ancient wheat varieties like Brkulja not only helps maintain agricultural biodiversity but also provides valuable genetic resources for developing healthier food products with enhanced bioactive properties. Future research should focus on identifying and quantifying the specific bioactive compounds responsible for the antioxidant activity of the Brkulja wheatgrass juice. Additionally, in vivo studies are needed to evaluate the bioavailability and physiological effects of these compounds in the human body. Finally, promoting the Brkulja wheat grass juice encourages the cultivation of a traditional, locally adapted wheat variety, which helps preserve agricultural biodiversity. Last but not the least, local processing and marketing of wheat grass juice can create jobs in rural communities, reducing urban migration and revitalizing village economies.

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Активност сока од пшеничне траве сорте Бркуља на инхибицији DPPH радикала и корелација између његових биоактивних једињења

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Сажетак

Истраживан је антиоксидантивни потенцијал сока од пшеничне траве добијен од старе сорте Бркуље како би се испитала његова функционална и нутрацеутска вриједност. Као стара аутохтона сорта, пшеница Бркуља показује генетску резилијентност и велику антиоксидативну и нутритивну вриједност што је чини важним извором биоактивних једињења. Пшенична трава је брана у раној фази влатања, а добијени сок је анализиран на укупни садржај фенола, концентрацију флавоноида и антиоксидативну активност употребом теста са циљем инхибиције DPPH радикала. Резултати су показали да сок од пшеничне траве сорте Бркуља посједује снажан антиоксидативни потенцијал уз вриједност IC_{50} од 0,75 μg/mL. Проценти инхибиције DPPH кретали су се од 88,83% при највишој концентрацији до 23,75% при најнижој концентрацији, показавши тако директну везу између концентрације сока и процента инхибиције. Укупни садржај фенола кретао се од 25,8 до 42,5 mg GAE/g, док је концентрација флавоноида била од 9,7 до 18,3 mg QE/g, чиме су показали јаку позитивну корелацију са инхибицијом DPPH (r > 0,9). Ови резултати иду у прилог употребе сока од пшеничне траве сорте Бркуља као природног извора антиоксиданса, чиме се истиче значај очувања старих сората пшенице и њихове примјене у здравствене сврхе.

Кључне ријечи: антиоксиданс, сок од пшеничне траве, полифеноли, здравствене предности.

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