Ordu Üniversitesi Bilim ve Teknoloji Dergisi
Ordu Üniv. Bil. Tek. Derg.

Ordu University Journal of Science and Technology
Ordu Univ. J. Sci. Tech.

E-ISSN: 2146-6459 2025, 15(2), 404-413 Araştırma Makalesi / Research Article
http://doi.org/10.54370/ordubtd.1812666

Evaluation of Bioactive Compounds and Phenolic Profiles of Pansy (*Viola* x *wittrockiana*) Cultivars Grown in Ordu Province

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Geliş Tarihi / Received Date: 28.10.2025 Kabul Tarihi / Accepted Date: 06.12.2025

Abstract

This study was conducted to determine some biochemical and bioactive compounds of four pansy (*Viola* x *wittrockiana*) cultivars: 'White with purple stain', 'Yellow with red stain', 'Violet yellow' and 'Red'. In this context, soluble solids content (SSC), titratable acidity (TA), vitamin C, total flavonoids, total phenols, antioxidant capacity (according to DPPH and FRAP), anthocyanins and individual phenolic compounds were analyzed. According to the findings, the 'Violet Yellow' pansy cultivar had the highest levels of SSC and vitamin C content. In terms of total flavonoids, total phenolics, antioxidant capacity and anthocyanin content, the 'Violet yellow' and 'Red' cultivars had higher values. In the evaluation of individual phenolic compounds, rutin was identified as the major phenolic compound in all cultivars. In conclusion, this study revealed that there are significant differences among edible pansy cultivars in terms of their biochemical and bioactive compound contents. The findings contribute to the potential use of pansy flowers in functional food ingredients and health-promoting products and show that variety selection is an important criterion.

Keywords: antioxidant, edible flower, ornamental plant, rutin, vitamin C

Ordu İlinde Yetiştirilen Hercai Menekşe Çeşitlerinin Biyoaktif Bileşenlerin ve Fenolik Profillerinin Değerlendirilmesi

Öz

Bu çalışma, 'White with purple stain', 'Yellow with red stain', 'Violet yellow' ve 'Red' hercai menekşe (*Viola* x *wittrockiana*) çeşitlerinin bazı biyokimyasal ve biyoaktif bileşiklerini belirlemek amacıyla yapılmıştır. Bu kapsamda, suda çözünür kuru madde (SÇKM), titre edilebilir asitlik (TEA), C vitamini, toplam flavonoid, toplam fenolik, antioksidan kapasitesi (DPPH ve FRAP testine göre), antosiyanin ile bireysel fenolik bileşik içerikleri analiz edildi. Elde edilen bulgular, 'Violet yellow' hercai menekşe çeşidinin SSC ve C vitamini içeriği bakımından en yüksek değerlere sahip olduğunu göstermiştir. Toplam flavonoid, toplam fenolik, antioksidan kapasite ve antosiyanin içerikleri açısından 'Violet yellow' ve 'Red' çeşitleri daha yüksek değerlere sahipti. Bireysel fenolik bileşikler açısında yapılan değerlendirmede ise rutin bileşiğinin tüm çeşitlerde majör fenolik bileşik olarak öne çıktığı görülmüştür. Sonuç olarak, bu çalışma yenilebilir hercai menekşe çeşitleri arasında biyokimyasal ve biyoaktif bileşik içerikleri açısından önemli farklılıkların bulunduğunu ortaya koymuştur. Elde edilen bulgular, hercai menekşe çiçeklerinin fonksiyonel gıda bileşenleri ve sağlığı geliştirici ürünlerde potansiyel kullanımına katkı sunmakta ve çeşit seçiminin önemli bir kriter olduğunu göstermektedir.

Anahtar Kelimeler: antioksidan, yenilebilir çiçek, süs bitkileri, rutin, vitamin C

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Introduction

Flowers have held a significant place in human history for their aesthetic appearance and symbolic meaning (Demasi et al., 2021). From ancient civilizations to the present day, many cultures have used flowers for both decorative and medicinal purposes in various ceremonies, festivals, and traditional events (González-Barrio et al., 2018; Kumari and Bhargava, 2021; Pensamiento-Niño et al., 2024).

In recent years, due to the increasing demand for natural products, there has been an interest in consuming flowers not only for decorative purposes but also for food. In this regard, due to the rise of epidemics, transportation and safety issues with drugs, and the potential side effects of synthetic compounds, products containing natural and safe bioactive compounds have gained importance (Fernandes et al., 2017; Lara-Cortés et al., 2013). In this context, flowers are among the natural resources that are gaining renewed value for both their aesthetic and functional properties.

Edible flowers have gained an important position in contemporary gastronomy and healthy eating habits thanks to both their decorative properties and potential bioactive compound content (Drava et al., 2020; Guiné et al., 2017; Koike et al., 2015; Mlcek & Rop, 2011). Flowers, especially rich in polyphenols, flavonoids, anthocyanins, and other phenolic compounds (Breda et al., 2025; Putpadungwipon & Powthong 2025), may have antioxidant and antimicrobial effects (Pumriw et al., 2025).

Edible flowers of ornamental plants are reported to be an important source of chemical compounds that exhibit antioxidant activity and have a marked inhibitory effect on reactive oxygen species (ROS) (Fu & Mao, 2008). Reactive oxygen species are, for example, hydroxyl radical, nitric oxide or superoxide anion, which are produced in the human body under stress conditions, high load conditions, various diseases, etc. (Castro & Freeman, 2001). For flowers, compounds with antioxidant activity are very important, especially because they inhibit the ageing process caused by the action of ROS on biomembranes (Panavas & Rubinstein, 1998). In this respect, edible flowers have attracted attention in recent years due to their positive effects on human health.

To utilize this potential effectively of edible flower, the biochemical and bioactive contents of these plants should be revealed on scientific basis. In addition, interspecific differences in the content and distribution of bioactive compounds Pinedo-Espinoza et al. (2020), as well as genetic differences due to varieties within the same species are also determinant. This situation makes it clear that detailed assessments are required not only at the species level, but also at the variety level.

In this context, pansy (*Viola x wittrockiana*), which is among the edible flowers, is a species that attracts attention with its aesthetic appearance and potential bioactive content. Especially the fact that it has different color variations increases the importance of studies aimed at revealing the contextual differences of these cultivars.

This study aimed to determine the biochemical and bioactive contents of pansy cultivars with different flower colors.

Materia and Method

Plant Material

Four pansy (*Viola x wittrockiana*) cultivars with different color characteristics were used as plant material. The cultivars are designated according to their flower color as ‘White with purple stain’ (C1), ‘Yellow with red stain’ (C2), Violet yellow’ (C3) and ‘Red’ (C4) (da Silva et al., 2020). Seedlings, approximately 10 cm tall, were obtained from a private nursery at the seedling stage. They were transplanted into 2-liter plastic pots containing peat and perlite (3:1, v/v) growing medium on May 15, 2023.

The experiment was carried out in a polycarbonate greenhouse at the experimental area of the Faculty of Agriculture, Ordu University (Ordu, Türkiye). The greenhouse was equipped with shading

system and roof ventilation, to maintain the temperature 25±2°C. During the cultivation, the plants were irrigated regularly with 300 mL of tap water every three days without fertilization or additional treatments.

A total of 120 pots were used 30 pots (10 pots x 3 replicates) for each cultivar, for. The growing medium was prepared in the same way for all cultivars. The experiment design followed a completely randomized design. Flowers were harvested when at least 5 flowers per plant were fully open. Twenty flowers were harvested from each replicate of each cultivar, totaling sixty flowers from one cultivar.

The harvested flowers were transported to the laboratory on the same day. Physicochemical analyses were performed on half of the flowers, while remaining flowers were stored at -24°C in falcon tubes for bioactive and individual phenolic analyses.

Method

Physicochemical Properties

Three replicates were created for each cultivar to determine Vitamin C, titratable acidity (TA) and soluble solids content (SSC) content. Analyses were performed on these replicates. A flower sample of 10 g from each replicate was mixed with 20 ml of distilled water to form a homogenate. To determine, a reflectoquant (Merck RQflex plus 10, Turkey) device and ascorbic acid test kit were used to determine vitamin C content. Results are presented as mg 100 g^{-1}.To determine the titratable acid content, 0.1 mol L^{-1} (N) sodium hydroxide (NaOH) was added to the homogenate and titration was performed. The amount of NaOH consumed until the homogenate reached pH 8.1 after the additions were determined, and calculations were made. The results are presented as g 100 g^{-1} citric acid. To determine SSC, the resulting content was determined dropwise by electronic refractometer (PAL-1, USA). The results are presented as percentages (Ates, 2023).

Bioactive Compounds

For the determination of total flavonoids content (TFC), total phenolic content (TPC), antioxidant capacity (according to DPPH and FRAP), total anthocyanins (TA), and individual phenolic compounds, 10 flowers were randomly collected from each replicate. A total of 30 flowers of one cultivar were used, corresponding to three replicates. Approximately 20 grams of flower material from each sample were placed in Falcon tubes and stored in a freezer at -80°C until the analysis was done. The samples were kept at room temperature (21°C) then were prepared for analysis [1/10 w/v]) according to the method described by Ates and Ozturk (2023). Antioxidant capacity was determined using two separate tests: the DPPH (r2,2-diphenyl-1-picryl-hydrazyl-hydrate) method, as described by Aglar et al. (2017), and the FRAP (Ferric Ions (Fe+3) Reducing-1 fw) assay, following the procedure by Benzie and Strain (1996). The antioxidant activities were expressed as Trolox equivalents (TE) FW. The total phenolics content was measured using Singleton and Rossi (1965) method, and the results were expressed as gallic acid equivalents (GAE). Total flavonoids content was determined using the method by Chang et al. (2002), and the values expressed as quercetin equivalents (QE). Anthocyanin content was determined using Giusti and Wrolstad (2001) the pH difference method of, and the values were expressed as cyanidin-3-glucoside equivalents (cy-3).

Individual Phenolic Compounds

In this study, the amounts of 4-hydroxybenzoic acid, 4-aminobenzoic acid, p-coumaric acid, caffeic acid, chlorogenic acid, ferulic acid, catechin and rutin in flower samples were determined. The flower samples removed from the refrigerator were disintegrated in liquid nitrogen and placed in a 50 mL falcon tube and then added with distilled water at a ratio of 3/1 (v/w). Then, the flower samples were homogenized for 5 min with vortex device. Afterwards, the supernatants were obtained by centrifugation at 9000 rpm 4°C for 10 min. The supernatants were passed through 0.45 filters and

transferred to 2 mL vials. The flower supernatants were then stored at -24°C until individual phenolic analysis.

The flowers samples were kept at room temperature and then prepared for analysis following the method described by Karakaya et al. (2021). The analyses were carried out using an Ultra-High Performance Liquid Chromatography (UHPLC) system equipped with a diode array detector (DAD-3000, USA). The values obtained were expressed as mg $100g^{-1}$ FW.

Statistical Analysis

The experiment, consisting of four cultivars with three replications and ten plants per replication, was conducted in a Completely Randomized Design (CRD). After testing for normality with the Kolmogorov-Smirnov test, the data were further tested for homogeneity of variance using the Levene test. The results were subjected to analysis of variance (ANOVA). To determine significant differences between varieties, the Tukey test for multiple comparisons was used with a significance level of $p < 0.05$. All statistical analyses were performed using SAS software (version 9.1, USA).

Results and Discussion

Physicochemical Properties

When the obtained data were evaluated, significant differences were found among the physicochemical contents of different pansy cultivars. These differences were particularly significant for soluble solids content (SSC) and vitamin C content. The C3 cultivar had significantly higher SSC content than all other varieties ($p < 0.05$). No statistically significant difference was found among the other cultivars in terms of SSC content. Regarding vitamin C content, C3 cultivar showed the highest value, while C1 had the lowest. C2 and C4 were found at similar levels in terms of vitamin C content. These two cultivars had significantly higher values than C1 but lower values than C3 ($p < 0.05$). In terms of titratable acidity, no statistically significant difference was observed among the cultivars (Table 1).

Table 1. Soluble Solids Content (SSC), Titratable Acidity (TA) and Vitamin C of Different Pansy Cultivars

Cultivars	SSC (%)	TA (g 100 g-1 citric acid FW.)	Vitamin C (mg 100g-1 FW.)
C1	8.0 ± 0.00 b	0.193 ± 0.00 a	15.6 ± 0.35 c
C2	8.5 ± 0.29 b	0.193 ± 0.02 a	22.7 ± 0.21 b
C3	10.0 ± 0.00 a	0.195 ± 0.00 a	27.8 ± 0.45 a
C4	8.0 ± 0.00 b	0.224 ± 0.02 a	22.9 ± 0.50 b

The difference between the means shown with the same lowercase letter in the same column is insignificant (Tukey, p<0.05). C1; White with purple stain', C2; 'Yellow with purple stain', C3; 'Violet yellow', C4; 'Red.

SSC and TA are generally known as harvest criteria for fresh produce (Uslu et al., 2024). In this respect, they are effective parameters in determining product edibility and consumer acceptability. Vitamin C is a bioactive compound that supports human health and is present not only in many fruits and vegetables, but also in flowers and petals (Dujmovic et al., 2022). The amounts of SSC, TA, and vitamin C can vary depending on the genetic basis of the species and environmental factors. In this context, Grzeszczuk et al. (2016) reported that the TA content of edible flowers of different species ranged from 0.107 to 0.814 % citric acid FW, While the L-ascorbic acid content ranged from 15.56 to 241.20 mg $100g^{-1}$ FW. In the same study, they measured the TA and L-ascorbic acid amounts of pansy flowers as 0.591 g 100 g^{-1} citric acid and 1.82 mg g^{-1} FW, respectively. According to these results, lower TA value was obtained in our study, whereas higher vitamin C contents were generally observed. Similarly, Pêgo et al. (2022), in their study on six violet cultivars, reported variations in SSC content between 2.33 and 5.33 ^{o}Brix and TA content ranging from 0.50 to 2.17%. Vieira (2013) reported the TA content of pansy flowers as 0.21 and 0.89 g of citric acid 100 g^{-1}, and SSC between

0.3 and 0.7 °Brix. Also, they reported that vitamin C content of pansy flowers was 255.96 mg 100 g^{-1}. Compared to previous studies, our results generally showed lower TA and vitamin C values, while SSC values were higher. These differences are likely due to cultivar characteristics, environmental conditions, and cultivation practices.

Bioactive Compounds

Statistically significant differences were determined among the bioactive compound contents of the pansy cultivars examined in the study ($p < 0.05$). In terms of total flavonoids content, cultivars C3 and C4 had significantly higher values compared to other cultivars. In contrast, C1 had the lowest total flavonoids content. Total flavonoids of C2 were higher than C1, but lower than those of C3 and C4. When total phenolic contents were evaluated, C3 and C4 had the highest value, while C1 had the lowest. The total phenols content of C2 was lower than C3 and C4, but higher than C1.

DPPH activity was highest in C3 and C4 and lowest in C1. In terms of FRAP activity, cultivars C3, C2 and C4 similarly reached the highest values, While C1 showed a significantly lower FRAP value compared to all other varieties ($p < 0.05$). Significant differences were also observed among the anthocyanin contents of all cultivars. The highest total anthocyanin content was measured in C4, while the lowest was found in C1. When the total anthocyanin contents of the cultivars were ranked from lowest to highest, they were as follows: C1 < C2 < C3 < C4 (Table 2).

Table 2. Bioactive Compounds of Different Pansy Cultivars

Cultivars	Total Flavonoids (mg QE 100g-1 FW)	Total Phenolics (mg GAE 100g-1 FW)	DPPH (mmol TE 100g-1 FW)	FRAP (mmol TE 100g-1 FW)	Anthocyanin (mg cy-3 GE 100g-1 FW)
C1	1012.6 ±20.4 c	1249 ± 18.9 c	26.2 ± 1.08 c	51.3 ± 3.14 b	2.41 ± 0.62 d
C2	1342.6 ± 18.1 b	1623 ± 34.2 b	33.6 ± 3.86 b	84.1 ± 2.43 a	4.90 ± 0.86 c
C3	1676.6 ± 11.3 a	1867 ± 34.6 a	44.9 ± 0.61 a	80.7 ± 0.47 a	9.24 ± 3.97 b
C4	1772.9 ± 44.2 a	1949 ± 42.9 a	40.9 ± 0.88 a	89.1 ± 3.38 a	17.89 ± 3.15 a

The difference between the means shown with the same lowercase letter in the same column is insignificant (Tukey, p<0.05). C1; White with purple stain', C2; 'Yellow with purple stain', C3; 'Violet yellow', C4; 'Red.

Compared to the total phenolic (0.31-235.50 mg chlorogenic acid equivalent g^{-1} DW) and flavonoids (7.67-89.38 mg rutin equivalent g^{-1} DW) ranges reported for ten different edible flowers by Xiong et al. (2014), the differently colored pansies in our study were exhibited higher levels of bioactive compounds.

Benvenuti et al. (2016) reported that antioxidant activity and anthocyanin content were higher in red and purple pansy flowers than in yellow and white flowers. Similarly, Skowyra et al. (2014) found that total phenolics, flavonoids, and anthocyanin contents were higher in red and purple varieties than in yellow flowers. Consistent with these studies, our results indicated that violet-yellow and red pansy cultivars generally had higher bioactive contents.

The colors diversity of edible flowers represents an important organoleptic property (Mlcek & Rop, 2011). While these properties are influenced by many chemical compounds, they are mainly influenced by carotenoid and anthocyanin contents. Anthocyanin levels in edible flowers have been reported to be related to total flavonoid levels (Friedman et al., 2010). The findings obtained in our study were found to be consistent with this statement. Indeed, anthocyanin amounts were measured higher in red and yellow violet cultivars, which have higher total flavonoid content, than in other cultivars.

Ksouri et al. (2009) and Mlcek and Rop (2011) reported that flavonoids and phenolic compounds are the most important plant compounds with antioxidant activity. Indeed, in our study, our results showed that flowers with higher antioxidant activity had higher total flavonoid, total phenolics, and total anthocyanin contents. Genetic suppression of anthocyanin and flavonoid biosynthesis is reported to result in pale or white flower coloration (Heller et al., 1985; Stich et al., 1992).

Accordingly, in our study, lower amounts of total flavonoids, total phenolics, and anthocyanins were obtained from white pansy cultivars, and they were also found to have lower antioxidant activity. In conclusion, it was observed that the antioxidant activity and bioactive compound contents of pansy flowers vary significantly depending on flower color and pigment density.

Individual Phenolic Compound

In this study, statistically significant differences were observed among the individual phenolic compounds contents of examined the pansy cultivars ($p < 0.05$). C1 had the highest aminobenzoic acid content, while C4 had the lowest. The aminobenzoic acid content of C2 was like that of C1 and C3 (Table 3).

Significant differences were also detected among all cultivars in hydroxybenzoic acid content ($p < 0.05$). C3 had the highest value, while C2 had the lowest. The hydroxybenzoic acid contents were ranked from lowest to highest as follows: C2 < C4 < C1 < C3. Similarly, statistically significant differences were identified among the cultivars in terms of ferulic acid content. The highest ferulic acid content was found in C4, and the lowest in C2. Therefore, the statistical ranking was as follows: C2 < C3 < C1 < C4. Chlorogenic acid content was highest in C4 ($p < 0.05$). While C1 and C3 had similar levels and the lowest contents, while C2 had a higher content than these two cultivars but lower than C4. In terms of caffeic acid content, C2 reached the highest value, while no statistically significant difference was observed among the other three cultivars. Epicatechin content was also significantly higher in C2 compared to the other cultivars, while the lowest content was detected in C4. C1 and C3 exhibited similar epicatechin levels.

Statistically significant differences were also found among the cultivars for both p-coumaric acid and rutin contents. C3 was found to have the highest values for both compounds. The lowest p-coumaric acid content was observed in C2 and C1, Also the lowest rutin content was detected in C2. C4 had higher p-coumaric acid levels than C2 and C1, but lower than C3. Rutin content in C4 was lower than in C3, but like C2 and C1. Furthermore, in this study, rutin was determined to be the major phenolics compound for pansy flowers across all cultivars (Table 3).

Table 3. Individual Phenolic Compound of Different Pansy Cultivars

Cultivars	Individual Phenolic Compounds (mg $100g^{-1}$ FW)							
	4-AA	4-HA	CJA	CFA	EPC	p-CA	t-FA	RT
C1	0.117 ± 0.01 a	0.587 ± 0.01 b	0.015 ± 0.00 c	0.019 ± 0.02 b	0.018 ± 0.01 b	0.769 ± 0.11 c	2.103 ± 0.24 b	16.51 ± 0.78 b
C2	0.077 ± 0.00 bc	0.369 ± 0.02 d	0.041 ± 0.01 b	0.300 ± 0.05 a	0.036 ± 0.08 a	0.638 ± 0.23 c	0.044 ± 0.00 d	12.25 ± 1.51 c
C3	0.103 ± 0.08 ab	0.641 ± 0.01 a	0.011 ± 0.00 c	0.017 ± 0.01 b	0.032 ± 0.23 ab	3.386 ± 0.32 a	0.139 ± 0.08 c	19.81 ± 1.11 a
C4	0.061 ± 0.01 c	0.499 ± 0.03 c	0.434 ± 0.01 a	0.017 ± 0.03 b	0.006 ± 0.01 c	1.721 ± 0.26 b	6.112 ± 0.87 a	14.82 ± 1.46 bc

The difference between the means shown with the same lowercase letter in the same column is insignificant (Tukey, p<0.05). C1; White with purple stain', C2; 'Yellow with purple stain', C3; 'Violet yellow', C4; 'Red. 4-AA; 4-Aminobenzoic acid, 4-HA; 4-Hidroxybenzoic acid, CJA; Chlorogenic acid, CFA; Caffeic acid EPC; Epicatechin, p-CA; p-Coumaric acid, t-FA, trans-Ferulic acid, RT; Rutin.

Phenolic acids are reported to play an important role in reducing oxidative stress in humans (Breda et al., 2025). Eight different phenolic acids were identified in the four different pansy cultivars examined in our study (Table 3). Socha et al. (2021) identified ferulic, caffeic, and p-coumaric acids in 14 different edible flower species and reported that begonia and marigold flowers contained six of the nine phenolic acids, with these species having the highest total phenolic acid content. Therefore, pansy flowers appear to be a flower rich in phenolic acids.

Caffeic acid is notable for its strong antioxidant and anti-inflammatory properties (Natarajan et al., 1996). In our study, the caffeic acid content ranged from 0.017 to 0.300 mg $100g^{-1}$, with the highest value determined in the yellow with purple stain cultivar. Breda et al. (2025) detected caffeic acid in only two marigold cultivars out of four different edible flower species and reported its amount as 71.61-101.10 µg g^{-1}. In the same study, epicatechin was detected only in *Rose germanium* flowers at a level of 6.49 µg g^{-1}, while in our study, it was found to range from 0.06-036 mg kg^{-1} in all pansy cultivars.

Breda et al. (2025) reported p-coumaric acid in the range of 6.32-24.72 µg g^{-1}, while Socha et al. (2021) reported p-coumaric acid in the range of 12.0-154.4 µg g^{-1}. In our study, p-coumaric acid varied between 0.769-3.386 mg $100g^{-1}$ in all cultivars. In addition, Breda et al. (2025) detected chlorogenic acid in only three flowers (14.11-37.15 µg g^{-1}), whereas in our study, chlorogenic acid was detected in all cultivars and the highest value was 0.434 mg $100g^{-1}$ the red colored cultivar. Socha et al. (2021) reported that hydroxybenzoic acid was found in only five species among 14 different edible flower species. However, in our study, hydroxybenzoic acid was detected in all pansy cultivars.

In conclusion, the phenolic acid profile determined in pansy flowers shows both diversities compared to other edible flowers reported in the literature and reveals that it is rich in functional components with high antioxidant potential.

Conclusions

This study revealed that there were significant differences between the biochemical and bioactive compound contents of edible “White with purple stain” (C1), “Yellow with purple stain” (C2), “Violet yellow” (C3) and “Red” (C4) pansy (*Viola* x *wittrockiana*) cultivars. The data obtained show that the C3 pansy cultivar stands out in terms of vitamin C content. While C1 cultivar had lower values in most parameters, C3 and C4 varieties reached higher levels in terms of total flavonoid, total phenolic matter, DPPH, FRAP and anthocyanin contents. This confirms the relationship between bioactive compounds and colour pigments. As a result of individual phenolic compound analyses, Among the individual phenolic compounds analyzed, rutin was found to be the main phenolic compound. Pansy cultivars C3 and C4 had a richer phenolic profile than the other cultivars.

These findings indicate that pansy varieties show intervarietal differences in terms of biochemical and functional properties and these differences may constitute an important criterion for the evaluation of the plant in functional food, natural antioxidant and health promoting products. Selection of cultivars with a high content of phenolic compounds can contribute to quality and functionality in product development. Therefore, further studies on the potential uses of pansy cultivars are recommended.

Ethics

There are no ethical issues with the publication of this article.

Conflict of Interest

There is no conflict of interest.

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