Research Article

International Journal of Agriculture and Wildlife Science

Uluslararası Tarım ve Yaban Hayatı Bilimleri Dergisi

2025, 11(2): 172 – 177, doi: 10.24180/ijaws.1704379

Effect of Aminoethoxyvinylglycine (AVG) as an Ethylene Inhibitor on Bioactive Compounds of Edible Pansy Flowers (*Viola × wittrockiana*)

Yenilebilir Hercai Menekşe Çiçeğinin (*Viola × wittrockiana*) Biyoaktif bileşikleri Üzerine Bir Etilen İnhibitörü Aminoethoxyvinylglycine (AVG)'in Etkisi

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Received: 22.05.2025 **Accepted:** 23.07.2025 **Published:** 29.08.2025

Abstract: Edible flowers are used by consumers as a dietary supplement in dishes such as salads, desserts, drinks and garnitures. They are foods that are not harmful to human health and have a high nutritional value. Edible flowers are foods with vitamins, minerals, phenolic compounds and high antioxidant capacity, and they add visual appeal and variety to dishes. In this study, the effect of the ethylene inhibitor aminoethoxyvinylglycine (AVG) on the bioaktive compounds of edible pansies was investigated. In the study, the treatments included control (distilled water), 250 µmol AVG, and 500 µmol AVG. The treatments were applied to the plants as a foliar spray. Measurements were made before and 5 days after application of AVG. In the study, no statistically significant difference was found between the treatments in terms of total phenolic content. However, the flavonoid content of flowers treated with AVG was found to be lower compared to the control treatment. On the other hand, total anthocyanin content significantly increased with AVG treatments. Indeed, the highest values were obtained with 250 µmol and 500 µmol AVG treatments, measuring 0.66 and 0.74 g cy-3 kg^{-1}, respectively. According to the DPPH test, the antioxidant activity of AVG-treated flowers was lower than that of the control group. However, the FRAP test showed that the 500 µmol AVG treatment significantly enhanced antioxidant activity. In the 250 µmol AVG treatment, catechin, chlorogenic acid, and epicatechin contents were found to be significantly higher compared to the other treatments, while in the 500 µmol AVG treatment, rutin content was determined to be significantly higher. As a result, the study revealed that AVG application has an effect on the phytochemical content of pansy flowers, and this effect varies according to the dose.

Keywords: Antioxidant, Flavonoid, Phenolics, Rutin, 4-hydroxybenzoic acid

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Öz: Yenilebilir çiçekler tüketiciler tarafından salata, tatlı, içecek ve garnitür gibi yemeklerde besin takviyesi olarak kullanılmaktadır. Ayrıca bunlar insan sağlığına zararlı olmayan ve besin değeri yüksek gıdalardır. Yenilebilir çiçekler vitamin, mineral, fenolik bileşikler ve yüksek antioksidan kapasiteye sahip gıdalardır ve yemeklere görsel çekicilik ve çeşitlilik katarlar. Bu çalışmada, etilen inhibitörü aminoetoksivinilglisinin (AVG) yenilebilir hercai menekşelerin biyoaktif bileşenleri üzerindeki etkisi araştırılmıştır. Çalışmada uygulama olarak kontrol (saf su), 250 µmol AVG ve 500 µmol AVG dozları kullanılmıştır. Uygulamalar, yapraktan püskürtme (sprey) yöntemi ile gerçekleştirilmiştir. Çalışmada, toplam fenolik içeriği bakımından uygulamalar arasında istatistiksel olarak anlamlı bir farkın olmadığı belirlenmiştir. Ancak, AVG uygulanan çiçeklerin flavonoid içeriğinin, kontrol grubuna kıyasla daha düşük olduğu tespit edilmiştir. Bununla birlikte, toplam antosiyanin içeriği AVG uygulamaları ile anlamlı düzeyde artmıştır. Nitekim en yüksek değerler sırasıyla 0.66 ve 0.74 g cy-3 kg^{-1} ile 250 µmol ve 500 µmol AVG uygulamalarında elde edilmiştir. DPPH testine göre, AVG uygulanan çiçeklerde antioksidan aktivite kontrol grubuna göre daha düşük bulunmuştur. Ancak, FRAP testine göre 500 µmol AVG uygulaması antioksidan aktiviteyi anlamlı şekilde artırmıştır. 250 µmol AVG uygulamasında, kateşin, klorojenik asit ve epikateşin içerikleri diğer uygulamalara kıyasla istatistiki açından önemli düzeyde daha yüksek bulunurken, 500 µmol AVG uygulamasında ise rutin içeriği önemli şekilde daha yüksek olduğu tespit edilmiştir. Sonuç olarak, çalışma AVG uygulamasının hercai menekşe çiçeklerinin fitokimyasal içeriği üzerinde etkili olduğunu ve bu etkinin doza göre değiştiğini ortaya koymuştur.

Anahtar Kelimeler: Antioksidan, Flavonoid, Fenolik, Rutin, 4-hidroksibenzoik asit

Cite as: Gun, S., Ozturk, B., & Ates, U. (2025). Effect of aminoethoxyvinylglycine (AVG) as an ethylene inhibitor on bioactive compounds of edible pansy flowers (Viola × wittrockiana). Uluslararası Tarım ve Yaban Hayatı Bilimleri Dergisi, 11(2), 172-177. doi: 10.24180/ijaws.1704379

Plagiarism/Ethic: This article has been reviewed by at least two referees and it has been confirmed that it is plagiarism-free and complies with research and publication ethics. https://dergipark.org.tr/tr/pub/ijaws

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INTRODUCTION

For centuries, flowers have been used by people to provide different aromas and colors to food and drinks. In recent years, interest in these flowers has increased again with the increase in the search for gastronomic experiences and the acceleration of the trend towards natural and healthy food sources (Rop et al., 2012; Fernandes et al., 2019).

The pansy (*Viola x wittrockiana*), which belonging to the Violaceae family, is a widely consumed edible flower with its visual appeal, ease of use in a variety of dishes from sweet to salty, and rich biochemical content (Lim, 2012; da Silva et al., 2020). Because of these features, edible flowers are increasingly used in salads, soups, syrups, appetizers, drinks and desserts (Villavicencio et al., 2018).

Pansy flowers contain various health-promoting compounds including, anthocyanins, flavonoids, carotenoids, potassium and phosphorus. It is has been reported that they have strong antioxidant and free radical scavenging properties (Peng et al., 2011; Rop et al., 2012).

Due to the presence of biochemical compounds such as phenolics, flavonoids, and antioxidants, pansy flowers are considered to play a beneficial and therapeutic role in human health. Indeed, various studies have demonstrated that antioxidants reduce cellular damage and exhibit anti-aging effects. Moreover, phenolic compounds have been shown to possess anti-inflammatory and anti-cancer properties (Fernandes et al., 2019; Al-Zughbi and Krayem 2022).

In recent years, the biochemical compound in edible flowers have attracted the attention of the food industry because they can be as natural alternatives to artificial additives and preservatives. In this respect, it is assumed that increasing with various applications will contribute to the increase in demand for these products.

Aminoethoxyvinylglycine (AVG) is a compound that can suppress ethylene production by inhibiting ACC synthesis, which has a physiological role in the biosynthesis of ethylene (Fadhil and Al-Bamarny, 2009). In this respect, it has been reported to effectively reduce product losses by delaying changes in biochemical properties of fresh products such as fruits and vegetables, including total phenolic, flavonoid and antioxidant activity (Kucuker et al., 2015; Yildiz et al., 2018).

This study investigated the effects of different AVG doses on the biochemical properties (such as total phenolic, total flavonoid, total monomer anthocyanin content and antioxidant activity as well as the individual phenolic components) of pansy flowers.

MATERIAL AND METHOD

Plant Materials

Pansy flowers (*Viola × wittrockiana*,'Viola Yellow' color) were obtained from pansies that had been cultivated in the greenhouse of Ordu University Faculty of Agriculture (Türkiye). Pansy plants were obtained as seedlings approximately 10 cm in height from a commercial company and were transplanted into the growing medium on June 1, 2023. The pansy plants were grown in 2-liter plastic pots filled with a growth medium composed of peat and perlite at a 3:1 (v/v) ratio. The plants were cultivated in a polycarbonate greenhouse where the temperature was maintained at 25 ± 2 °C using roof ventilation and shading systems. During the cultivation period, the plants were irrigated regularly every two days, and no fertilizer was applied.

Treatments

AVG was applied to the plants at two different concentrations (250 and 500 μmol). It was first dissolved in trace amounts of alcohol and then diluted to the desired concentrations using distilled water. In the control treatment, only distilled water was used. Three treatments were applied in total: control (distilled water), 250 μmol AVG, and 500 μmol AVG. All treatments were applied as a foliar spray until the plants were thoroughly wetted. Spray applications were performed 15 days after planting, during the stage when all plants exhibited homogeneous growth, and each plant had approximately 5 ± 1 flowers.

For measurements, the flowers were harvested before and 5 days after the application. In this respect, the values of bioactive components of flower samples are given as harvest and harvest + 5th day. In all harvesting periods, the flowers were harvested in a completely open stage. Flowers were then disintegrated in liquid nitrogen and then were refrigerated at -80°C until biochemical analysis.

Bioactive Compound Analysis

For measurement of the total phenolics, antioxidant capacity, total anthocyanin and individual phenolic components, 5 flower were harvested randomly from each repetition. Approximately 10 g of the flower was stored in a deep freezer at −80°C in falcon tubes until analysis was made.

Total phenolics content were measured following the protocol established by Singleton and Rossi (1965) and was expressed as grams of gallic acid equivalent (GAE) per kilogram of fresh weight (fw). Total flavonoids were determined by using the method of Chang et al. (2002) and was expressed as grams of quercetin equivalent (QE) per kilogram fw. The antioxidant capacity was evaluated using two different procedures of 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) described by Aglar et al. (2017) and Ferric Ions (Fe+3) Reducing-1 fw (FRAP) described by Benzie and Strain (1996). Antioxidant activity were reported as millimole of trolox equivalent (TE) per kilogram fw. Total monomeric anthocyanins of pansy flowers were measured according to the pH difference method as described by Giusti and Wrolstad (2001) and the results were expressed in g cy-3 kg^{-1} fw.

Individual Phenolic Compounds

In this study, the content of individual phenolic compounds such as, p-coumaric acid, rutin, chlorogenic acid, protocatechuic acid, caffeic acid, ferulic acid, catechin, 4-aminobenzoic acid and 4-hydroxybenzoic acid was determined in pansy flowers. Chromatographic separation was performed using an UHPLC (ultra-high performance liquid chromatography system) equipped with a diode array detector (DAD-3000, USA). Individual phenolic compounds were measured according to the method described by Karakaya et al. (2021). The results were expressed in mg kg^{-1} fw.

Statistical Analysis

Descriptive statistics were calculated for the data that met the necessary assumptions, and these data were evaluated using analysis of variance (ANOVA). Following ANOVA, the significance of differences among treatments was determined using Tukey's multiple comparison test. Statistical analyses were performed using the MINITAB 17 software package. A significance level of $\alpha = 0.05$ ($p < 0.05$) was considered in all analyses and interpretations.

RESULTS AND DISCUSSION

In the study, it was determined that the total phenol content of pansy flowers increased in harvest +5th day compared to harvest day. However, there was no statistically significant difference between the control and AVG treatments (250 µmol and 500 µmol). The findings in our study were similar to those of some previous studies. Indeed, Yildiz et al. (2018) and Karaman et al. (2013) reported that pre-harvest AVG treatment was not effective on change of the phenolic contents of sweet cherry and plum fruits.

Table 1. Effect of AVG treatments on total phenolic, total flavonoid and total monomerik anthocyanins contents of pansy flowers.

Çizelge 1. AVG uygulamalarının hercai menekşe çiçeklerinin toplam fenolik, toplam flavonoid ve toplam monomerik antosiyanin içerikleri üzerine etkisi.

Treatments	Total Phenolic (g GAE kg^{-1})		Total Flavonoid (g QE kg^{-1})		Total monomeric anthocyanins (g cy-3 kg^{-1})	
	Harvest	Harvest + 5th day	Harvest	Harvest + 5th day	Harvest	Harvest + 5th day
Control	2.33	2.92 ± 0.11 a	9.73	9.63 ± 0.09 a	0.52	0.53 ± 0.01 c
250 µmol AVG	2.33	2.87 ± 0.01 a	9.73	7.55 ± 0.09 b	0.52	0.74 ± 0.00 a
500 µmol AVG	2.33	3.02 ± 0.02 a	9.73	7.27 ± 0.26 b	0.52	0.66 ± 0.04 b

Values are presented as mean ± SD (n = 3). Different lowercase letters in the same column indicate significant differences according to Tukey's test ($p < 0.05$).

Total flavonoid content of pansy flowers decreased at harvest +5th days. On the other hand, in harvest +5th days, a statistically significant difference was detected between the control and AVG treatments. Indeed, the flavonoid content in AVG-treated flowers was significantly lower than the control treatment.

Anthocyanin analysis showed that AVG treatment increased the anthocyanin content of pansy flowers. The highest anthocyanin content was recorded in the 250 μmol AVG treatment (0.74 mg cy-3 kg^{-1}), while the lowest content was observed in the control treatment (0.53 mg cy-3 kg^{-1}). Similar to our results, Babalık (2021) reported that AVG increased the anthocyanin content in grapefruit. In contrast, Yildiz et al. (2018) found that lower anthocyanin content was measured in the AVG treatment. It has been reported that AVG may adaptively increase anthocyanin synthesis by increasing stress-responsive signalling pathways in some species (Babalık, 2021). It is thought that our study may have involved a similar mechanism, which could explain the observed increase in anthocyanin content.

Table 2. Effect of AVG treatments on antioxidant activities (DPPH and FRAP analysis) of pansy flowers.
Çizelge 2. AVG uygulamalarının hercai menekşe çiçeklerinin antioksidant aktivitesi (DPPH ve FRAP) üzerine etkisi.

Treatments	DPPH· (mmol TE kg^{-1})		FRAP· (mmol TE kg^{-1})	
	Harvest	Harvest + 5th day	Harvest	Harvest + 5th day
Control	374	660 ± 1.43 a	879	814 ± 25.7 b
250 μmol AVG	374	438 ± 28.1 c	879	778 ± 29.1 b
500 μmol AVG	374	612 ± 12.0 b	879	907 ± 25.1 a

Values are presented as mean ± SD (n = 3). Different lowercase letters in the same column indicate significant differences according to Tukey's test ($p < 0.05$).

When the antioxidant activity data were examined, it was determined that AVG treatments reduced antioxidant activity according to DPPH analysis. Specifically, the highest antioxidant activity (660 mmol TE kg^{-1}) was obtained in the control treatment while the lowest (438 mmol TE kg^{-1}) was obtained in the 250 μmol AVG treatment. According to FRAP test, the highest antioxidant activity was recorded in the 500 μmol AVG treatment, whereas the lowest was again observed in the 250 μmol AVG treatment. Similar to our findings, some previous studies reported that the antioxidant activity of sweet cherry (Yildiz et al., 2018) and plum (Karaman et al., 2013) fruit treated with pre-harvest AVG was lower compared to the control. In contrast, Aglar (2023) reported that AVG treatment increased antioxidant activity in jujube fruits. Khan et al. (2007) suggested that AVG delays ripening in some fruit species by suppressing ethylene synthesis and thus reducing the biosynthesis of antioxidant compounds. In this context it is presumed that AVG treatment in our study delayed flowers maturation leading to decrease in antioxidant activity.

Table 3. Effect of AVG treatments on individual phenolic compounds of pansy flowers.
Çizelge 3. AVG uygulamalarının hercai menekşe çiçeklerinin bireysel fenolik bileşikleri üzerine etkisi.

Treatments	Individual phenolic compounds (mg kg^{-1})			
	Aminobenzoic	Protocatechuic	Hydroxybenzoic	Catechin
Control	0.35 ± 0.00 a	5.01 ± 0.03 b	176 ± 3.33 a	5.58 ± 0.02 c
250 μmol AVG	0.59 ± 0,20 a	6.34 ± 0.02 a	111 ± 0.80 c	9.95 ± 0.04 a
500 μmol AVG	0.79 ± 0,23 a	6.45 ± 0.15 a	152 ± 1.07 b	7.62 ± 0.02 b
	Chlorogenic	**Caffeic**	**Epicatechin**	**Rutin**
Control	2.57 ± 0.01 b	13.1 ± 0.04 a	166 ± 0.12 b	1661 ± 1.05 c
250 μmol AVG	5.33 ± 0.31 a	7.8 ± 0.05 b	203 ± 2.00 a	1816 ± 3.96 b
500 μmol AVG	2.39 ± 0.02 b	11.8 ± 1.30 a	116 ± 1.00 c	1968 ± 6.96 a

Values are presented as mean ± SD (n = 3). Different lowercase letters in the same column indicate significant differences according to Tukey's test ($p < 0.05$).

As a result of the individual phenolic compound analyses, aminobenzoic acid, protocatechuic acid, hydroxybenzoic, catechin, chlorogenic acid, caffeic acid, epicatechin and rutin were detected in pansy flower. It was determined that AVG treatments had varying effect on the levels of some individual phenolic compounds depending on the treatments dose. Indeed, the amounts of catechin, chlorogenic acid, and epicatechin were significantly higher in the 250 μmol AVG treatment compared to the other treatments.

Rutin content was higher in the 500 µmol AVG treatment. Hydroxybenzoic acid content was significantly higher in the control treatment, while caffeic acid content was found to be similar between the control and 500 µmol AVG treatments.

CONCLUSION

AVG treatments exhibited varying effects on different bioactive compounds. AVG treatments had no effect on the phenolic content of pansy flowers. However, they were found to reduce flavonoid content while enhancing anthocyanin levels. The effect of AVG treatments on antioxidant activity differed according to the test. Specifically, AVG treatments reduced antioxidant activity according to DPPH assay, While high AVG dose increased antioxidant activity according to FRAP assay. In conclusion, the study recealed that AVG treatment influenced the phytochemical compound of edible pansy flower and this effect varied according to the dose.

CONFLICT OF INTEREST

S. Gun, B. Ozturk and C. A. Hekimoglu Has declare that they have no competing interests.

DECLARATION OF AUTHOR CONTRIBUTION

The authors contributed equally to the article.

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