RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(3): 690-695
DOI: https://doi.org/10.31015/jaefs.2023.3.22

Effect of phosphorus fertilization on phenolic compounds and antioxidant activity in *Galanthus elwesii* Hook.

Ebru Bati Ay[1] • **Sevket Metin Kara**[2] • **Muhammed Akif Açikgöz**[3]

[1] Suluova Vocational School, Amasya University, Amasya, Türkiye
[2] Faculty of Agriculture, Ordu University, Ordu, Türkiye
[3] Faculty of Agriculture, Ordu University, Ordu, Türkiye

Citation: Bati Ay, E., Kara, S.M., Acikgoz, M.A. (2023). Effect of phosphorus fertilization on phenolic compounds and antioxidant activity in Galanthus elwesii Hook. International Journal of Agriculture, Environment and Food Sciences, 7 (3), 690-695

Received: August 24, 2023
Revised: September 11, 2023
Accepted: September 13, 2023
Published Online: September 29, 2023

Corresponding Author: Ebru Bati Ay
E-mail: ebru.bati@amasya.edu.tr

Available online at
https://jaefs.com/
https://dergipark.org.tr/jaefs

Abstract

Snowdrop is a genus of high medicinal value with alkaloids such as galantamine, and lycorine of the *Amaryllidaceae* family. The present study was conducted to have an effect on the effects of phosphorus (P) treatment on antioxidant activity and phenolic compounds in *Galanthus elwesii* Hook. The plants were exposed to different concentrations of P (0, 3, 6, and 12 kg da^{-1}). The study was carried out in the 2018-2019 growing season. *G. elwesii* were harvested based on different growing stages (flowering and fruit ripening). In this study, the bulb and roots of the plant were used. Total flavonoid content (TFC), total phenolic content (TPC), phenolic compounds, and antioxidant activity were determined in harvested bulb and roots. The highest TPC was detected as 358.36 mg GAE/g in the flowering period of the plant, and the lowest TPC determined as 80.13 mg GAE/g in the fruit ripening period in the treatment P 6 kg da^{-1}. The highest TFC was detected as 108.07 mg QE/g with the flowering period of the plant, and the lowest TFC was determined as 52.33 mg QE/g in the fruit ripening period in the treatment P 6 kg da^{-1}. The main phenolic component of *G. elwesii* was determined to be gallic acid (GA). In antioxidant activity, while the highest 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity (72.30%) was in the flowering period in the treatment P 6 kg da^{-1}, the highest ferrous ions chelating activity (66.77%) was detected in the fruit ripening period in the treatment P 6 kg da^{-1}. As a result, it was determined that TPC, TFC and DPPH activity in *G. elwesii* of flowering period >fruit ripening period.

Keywords: *Galanthus elwesii,* Phosphorus, Alkaloids, Blbous plants, Growth and development periods

INTRODUCTION

Snowdrop is among the most significant species of the *Amaryllidaceae* family (Semerdjieva et al., 2019). The species in the *Amaryllidaceae* family contain up to 150 alkaloids called *Amaryllidaceae* alkaloids such as nivalin, galantamine, tazettin, and lychorenin, which have high biological activities, and according to their structures, they also have anticancer (Ay et al., 2023), anti-inflammatory (Kang et al., 2012), anti-diabetic (Ghane et al., 2018), and anti-bacterial, anti-malarial, acetylcholinesterase and butyrylcholinesterase inhibition (Pesaresi et al., 2022; Ay et al., 2023) effects. The active agents of snowdrop species, minimize the damage they cause to the body by blocking free radicals and have antioxidant effects that prevent chain reactions that lead to premature aging as well as many diseases. Natural antioxidants that are common in plants are phenolic compounds, nitrogen compounds, polyphenols, carotenoids, ascorbic acid, and selenium (Fernandez-Lopez et al., 2020). The antioxidant activity of phenolic

compounds was investigated by many researchers and the structural characteristics of flavonoids, which are among the compounds providing antioxidant activity, were identified (Deveci et al., 2018). Flavonoids are phenolic compounds among the most common groups of secondary metabolites in various food and medicinal plants.

Factors that cause stress in the medicinal plant may be of living origin such as disease-causing agents and pests, or may also be of inanimate origin such as salinity, drought, low and high temperatures, radiation, and deficiencies or excesses of nutrients (Taiz and Zeiger, 2010). The deficiency or excess of a nutrient element may have positive or negative effects on the availability and toxicity of another nutrient element to the plant, which may create a stress effect in terms of plant development. The effects of increasing doses of macronutrients on plant growth in some medicinal and aromatic plants and especially on secondary metabolites have recently become increasingly important. P is an essential macronutrient that plays a role in many physiological processes such as cell division nucleic acid synthesis, , membrane stability, respiration, enzymatic activities, photosynthesis and development in plants (Orme˜no and Fernandez, 2012; Abbas et al., 2018; Kalayu, 2019).

Although the medicinal value of the *Amaryllidaceae* family is already known, the number of studies conducted on the antioxidant capacity, and total phenolic and flavonoid contents of this plant is quite a few. No studies were detected in the literature regarding the effects of fertilizer applications on total phenolic and flavonoid contents and antioxidant capacity, especially in snowdrop.

In light of these, the purpose of this research investigate the effects of P on phenolic compounds and antioxidant activity in *G. elwesii*.

MATERIALS AND METHODS

Material and design

The study was conducted in the 2018-2019 growing seasons in a land previously used as agricultural land in the Suluova district of Amasya. In the study, *G. elwesii* species with a bulb diameter larger than 4 cm, obtained from commercial companies, was used. The study was created in the "Randomized Complete Block Design" trial design with 3 replications. Three different concentrations of P (0, 3, 6, and 12 kg da^{-1} P_2O_5 - 0.43% w/v) were applied. After the drying process was completed, the root and bulbs were macerated.

Phytochemical analysis

Preparation of plant samples to determine antioxidant activity

After the drying process was completed, the plants were macerated. Then the methanol was evaporated by rotary and the extracts were obtained.

Determination total phenolic content (TPC)

TPC of the *G. elwesii* plant extracts was found according to the method suggested by Slinkard and Singleton (1977), using. The results are expressed as mg gallic acid/g (mg GAE/g) in the dried samples.

Determination total flavonoids content (TFC)

TFC was determined with the quercetin standard solution by using the methods suggested by Park et al. (2008) and total flavonoids were expressed as mg equivalents of quercetin (mg QE/g) per g of the dried fraction.

Determination phenolic compounds

The amounts of gallic acid, caffeic acid, campherol, formonenitin, p-coumaric, cinnamic, and ferulic acid were determined with high-performance liquid chromatography (HPLC).

Antioxidant activity

DPPH Free Radical Scavenging Activity

In the present study, DPPH free radical scavenging method was used to determine the antioxidant activity (Brand-Williams et al., 1995).

Ferrous ions chelating assay

The chelating activity (Fe^{2+}) of the extracts in iron ions was determined according to the method proposed by Decker and Welch (1990).

Statistical Analysis

Evaluation of the obtained data was done by using JUMP statistical package program. The significance control of the differences between the means was performed using the Duncan test.

RESULTS AND DISCUSSION

Total phenolic content

TPC founded from *G. elwesii* samples treated with P fertilizer are shown in Figure 1. The highest TPC during the flowering period was found to be 358.36 mg GAE/g with 6 kg/da P treatment. The lowest TPC during fruit ripening period was found to be 80.13 mg GAE/g with 6 kg/da P treatment. When the related figure is examined, it is seen that the total phenolic content increased by 117.12% in the 6 kg/da P treatment during the flowering period.

Ay et al., (2018) conducted a study to determine the total phenolic content of *G. elwesii* . The highest phenolic content in *G. elwesii* extracts was found to be 42.63 mg GAE/g in the fruit ripening period, the lowest phenolic content was determined as 18.15 mg GAE/g in the fruit ripening period. In the study of Korcan et al. (2018) in which they determined the phenolic compounds, total flavonoid substance, antioxidant capacity, and

antimicrobial activity in bulbs of a narcissus species *Narcissus papyraceus*, the amount of total phenolic substance in bulbs and bulbs skins was determined as gallic acid equivalent of 98 mg GAE/g and 584 mg GAE/g, respectively. Erenler et al., (2019) conducted a study to determine the antioxidant activity and total phenolic content of *Galanthus krasnovii*. The total phenolic contents of dichloromethane- ,hexane-, and ethyl acetate extracts were 60.95 mg GAE/g, 71.90 GAE/g, and 58.90 mg GAE/g, respectively. Albayrak and Elmacı (2017) found that the effect of increasing nitrogen and sulfur doses on the total phenolic substance and antioxidant activity in bulbs was insignificant, and the total phenolic substance content was 63.16 mg 100 g^{-1} and 76.92 mg 100 g^{-1}.

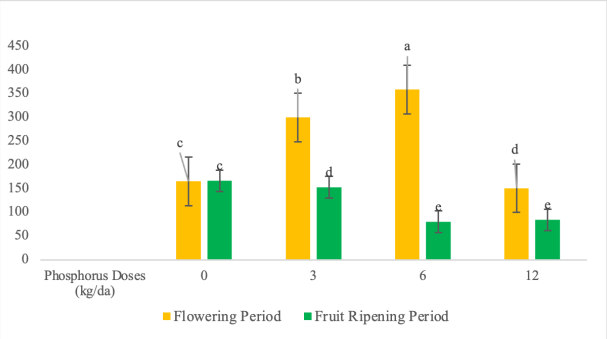

Figure 1. Total phenolic substance content (mg GAE/g) values of *G. elwesii* plants treatment with different doses of phosphorus (P) fertilizer according to different development periods.

Total flavonoid content

TFC obtained from *G. elwesii* samples treated with P fertilizer are shown in Figure 2. The highest TFC during the flowering period was found to be 108.07 mg QE/g with 6 kg/da P treatment. The lowest TPC during fruit ripening period was found to be 52.33 mg mg QE/g with 6 kg/da P treatment. As seen in Figure 2, the total flavonoid content of *G. elwesii* plant decreased by 51.57 % in the fruit ripening period compared to the flowering period.

Ay et al. (2018) reported that the highest flavonoid content in *G.elwesii* extracts was determined in the fruit ripening period, and the lowest flavonoid content was found in the the fruit ripening period. Korcan et al. (2018) determined the total flavonoid content in *Narcissus papyraceus* bulbs and bulb skins as 8.75 mg QE/g sample and 5.04 mg QE/g sample as quercetin equivalents, respectively. In a study that was conducted by Deniz (2016), when the flavonoid amounts of *Crocus* L. taxa extracts were compared, although the highest amount was found in *C. cancellatus* subsp. as 60.71 mg QE/g in the ethanolic areial extract of the mazziaricus taxon, the lowest value was in *C. pallasii subsp*. Pallasii taxon as 5.65 mg QE/g in the ethanolic underground extract. This study results support previous study results.

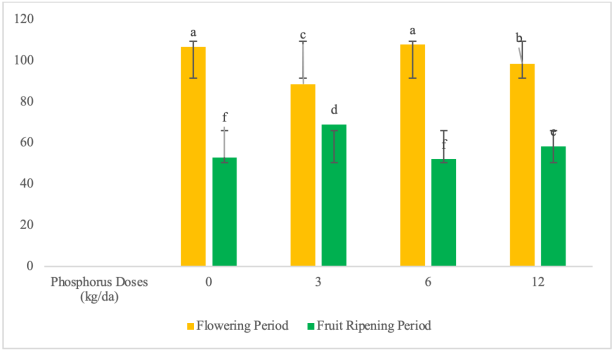

Figure 2. Total flavonoid content (mg QE/g) values of *G. elwesii* plants treatment with different doses of phosphorus (P) fertilizer according to different development periods.

Determination phenolic compounds

The phenolic substances identified were caffeic acid, gallic acid, p-coumaric acid, ferulic acid, canferol, cinnamic acid, syringer, vanillic acid and formonentin. Only gallic acid and caffeic acid were detected in the bulb and root that were harvested during flowering and fruit ripening periods, but p-coumaric acid, ferulic acid, cinnamic acid, syringer, canferol, formonentin, and vanillic acid could not be detected. When the amount of gallic acid was evaluated, it was found that the amount of gallic acid in the fruit ripening period ranged between 167.39-313.03 µg/ml. Although the amount of gallic acid in the underground organs during the flowering period varied between 153.84-1039.78 µg/ml. During the flowering period, the amount of caffeic acid was found to be 0.04-0.09 µg/ml in the bulb and root. Caffeic acid could not be detected in the bulb and root organs during the flowering period. Studies conducted on the determining phenolic compounds with this species were very limited. In the phenolic component determination study of *Galanthus elwesii*, Ay et al., (2018) found gallic acid, caffeic acid, myricetin, kaemferol, formononetin, and quercetin. Prakash et al., (2007) determined phenolic compounds such as gallic acid, ferulic acid, protocatechic acid, quercetin and campherol in their study conducted for HPLC and LC-MS/MS in four types of bulbs (red, purple, white, and green). At the end of their study, they found that the amount of ferulic acid varied between 13.5 and 116, the amount of gallic acid between 9.3 and 354, the amount of protocatechic acid between 3.1 and 138, the amount of quercetin between 14.5 and 5110, and the amount of campherol between 3.2 and 481 µg/g. In the present study, it was found that the main phenolic component of *G. elwesii*, which was applied at different doses of P, was gallic acid.

Antioxidant activity

DPPH free radical scavenging activity obtained from *G. elwesii* samples treated with P fertilizer are shown in Figure 3. The highest DPPH free radical scavenging

activity during the flowering period was found to be 72.30% with 6 kg/da P treatment. The lowest DPPH free radical scavenging activity during fruit ripening period was found to be 49.85% with 3 kg/da P treatment. As seen in Figure 3, DPPH free radical scavenging activity of *G.elwesii* plant decreased by 53.05% in the fruit ripening period compared to the flowering period.

Ferrous ions chelating assay obtained from *G. elwesii* samples treated with P fertilizer are shown in Figure 4. The highest ferrous ions chelating assay during the fruit ripening period was found to be 66.77% with 6 kg/da P treatment. The lowest ferrous ions chelating assay during the flowering period was found to be 50.55% with 12 kg/da P treatment. As seen in Figure 4, DPPH free radical scavenging activity of *G.elwesii* plant decreased by 24.29% in the the flowering period compared to fruit ripening period.

Antioxidant studies on *Galanthus* species are very limited. As a result of our study, the DPPH ranking was found as fruit ripening period <flowering period according to the harvest time in the bulbs and roots. Aydin et al., (2015) reported that ethanol extracts obtained from bulbs and leaves of *Sternbergia lutea* species had higher antioxidant activity than bulbs extracts (86.60%) and leaf extracts (68.10%). Deniz (2016) investigated two plant taxa of the genus *Crocus* L., which are in the *Iridaceae* family, including very important geophytes in terms of chemical contents, and spreads in Denizli. The highest total antioxidant activity was detected in *C. pallasii subsp*. pallasii taxon, the underground extract (90.25%), the highest free radical scavenging activity was detected in the underground extract of the *C. cancellatus* subsp. mazziaricus taxon prepared with acetone (90.3%). Turan (2016) conducted a study to determine the antioxidant activities, phenolic substances, and flavonoids, the phenolic components with spectroscopic methods in the *Cyclamen alpinum Dammann* ex. *Sprenger* and *Cyclamen parviflorum Pobed*. In antioxidant activity trials, the highest activity was detected in the areial parts part of *C. parviflorum* (91.39%), but the lowest activity was detected in the underground part of *C. alpinum* (13.11%). Korcan et al., (2018) performed a study to determine the phenolic compound, total flavonoid substance, and total antioxidant capacity and antimicrobial activity in *Narcissus* Species *Narcissus papyraceus* bulbs grown in Izmir. The remaining DPPH rank of ethanol extracts of the plants at 30 µg/ml plant concentration is bulb peels (88.96%) > bulbs (55.5%). In the plant extracts that were prepared with water, the remaining DPPH rank of 30 µg/ml concentration was bulb peels (66.1%) > bulbs (25.3%). In a study conducted by Ay et al. (2018) to determine antioxidant activity of *G. elwesii* in flowering period and fruit ripening period, and reported that the highest DPPH free radical scavenging activity was in the fruit ripening period of *Galanthus elwesii* extracts. It was reported in the same study that antioxidant activity showed significant differences according to growth periods and plant organs.

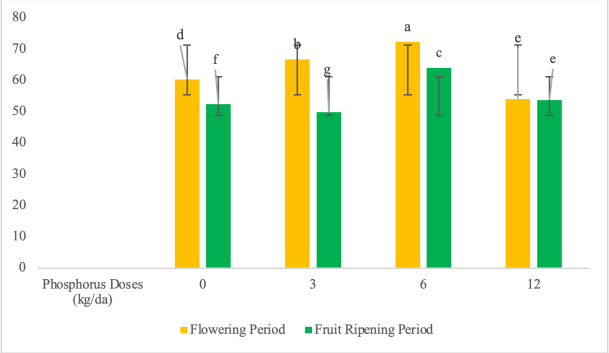

Figure 3. DPPH free radical scavenging activity (%inhibisyon) values of *G. elwesii* plants treatment with different doses of phosphorus (P) fertilizer according to different development periods.

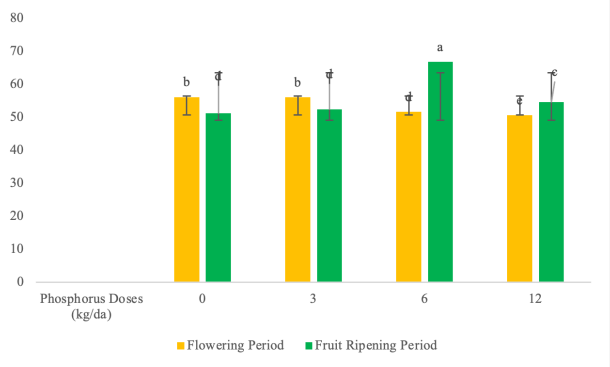

Figure 4. Ferrous ions chelating assay (%inhibisyon) values of *G. elwesii* plants treatment with different doses of phosphorus (P) fertilizer according to different development periods.

CONCLUSION

In the present study, the highest amount of total phenolic was determined as 358.36 mg gallic acid/g in *G. elwesii* species during the flowering period of the plant, and the lowest total phenolic content was determined as 80.13 mg gallic acid/g in the fruit ripening period. The highest amount of total flavonoid content was determined in the flowering period of the plant. Although it was found as 108.07 mg QE/g, the lowest total flavonoid content was determined as 52.33 mg QE/g in the fruit ripening period with 6 kg/da P treatment. gallic acid was the major phenolic component of *G. elwesii*. The highest DPPH free radical scavenging activity in the *G. elwesii* was determined to be 72.30% with 6 kg/da P treatment during the flowering period of the plant, while the lowest DPPH free radical scavenging activity was detected as 49.85% with 3 kg/da P treatment in the fruit ripening period of the plant. The highest ferrous ions chelating assay during the fruit ripening period was found to be

66.77% with 6 kg/da P treatment. The lowest ferrous ions chelating assay during the flowering period was found to be 50.55% with 12 kg/da P treatment. When the *G. elwesii* is evaluated according to different harvest times, it is seen that the highest total flavonoid , total phenolic content and DPPH free radical scavenging activity are in the flowering period. Only the amount of ferrous ions chelating assay was determined to be the highest during the fruit ripening period, which shows that the amount of secondary metabolite will vary according to the organ in the plant, harvest time, and many factors. In the present study, it is possible to express the total flavonoid , total phenolic content and DPPH free radical scavenging activity fruit ripening period <flowering period.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution

Ebru Batı Ay: Investigation, Project administration, Writing, Funding acquisition; Şevket Metin Kara: Supervisor, Editing; Muhammed Akif Açıkgöz: Editing.

All the authors verify that the text, figures, and tables are original and that they have not been published before.

Ethics committee approval

Ethics committee approval is not required. This article does not contain any studies with human participants or animals performed by any of the authors.

Funding

We would like to thank the Scientific Research Projects Unit (BAP) of Ordu University for providing support to this research, as a part of the TF-1645 BAP Project.

Data availability

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Acknowledgments

This study is a part of Dr. Ebru BATI AY's doctorate thesis.

REFERENCES

Abbas, G., Chen, Y., Khan, F.Y., Feng, Y., Palta, J.A., Siddique, K.H., (2018). Salinity and low phosphorus differentially affect shoot and root traits in two wheat cultivars with contrasting tolerance to salt. Agronomy 8 (8), 155. https://doi.org/10.3390/agronomy8080155

Albayrak, B., & Elmacı, Ö. L., (2017). Azot ve Kükürtlü Gübre Uygulamalarının Kuru Soğanda (*Allium cepa* L.) C Vitamini, Antioksidan Aktivite ve Toplam Fenolik Madde Miktarlarına Etkileri. Adnan Menderes üniversitesi Ziraat Dergisi, 14 (2):1-5.

Ay, B. E., Gül, M., Açikgöz, M.A., Yarilgaç, T., & Kara, Ş. M. (2018). Assessment of Antioxidant Activity of Giant Snowdrop (*Galanthus elwesii* Hook) Extracts with Their Total Phenol and Flavonoid Contents. Indian Journal of Pharmaceutical Education and Research, 52(Issue 4 (Suppl), 128-132.

Batı Ay, E. (2019). Farklı Fosfor ve Çinko Dozları Uygulanan Kardelende (*Galanthus elwesii* Hook.) Fenolik Bileşikler, Alkaloit İçeriği ve Antioksidan Aktivitenin Bitki Organlarına ve Gelişme Dönemlerine Göre Değişimi.

Brand-Williams W, Cuvelier ME, Berset C, (1995). Use of a Free Radical Method to Evaluate Antioxidant Activity. LWT-Food Sci. Techno., 28, 25–30.

Ay, E. B., Açıkgöz, M. A., Kocaman, B., Mesci, S., Kocaman, B., Yıldırım, T. (2023). Zinc and phosphorus fertilization in Galanthus elwesii Hook: Changes in the total alkaloid, flavonoid, and phenolic content, and evaluation of anti-cancer, anti-microbial, and antioxidant activities. Scientia Horticulturae, 317, 112034.

Aydın, Ç. (2016). Bazı geofit ekstraktlarının farklı biyolojik aktivitelerinin araştırılması. Doktora Tezi, Pamukkale Üniversitesi, Fen Bilimleri Enstitüsü, Denizli.

Deniz, N. (2016). *Crocus cancellatus* Herbert subsp. mazziaricus (Herbert) Mathew ve *Crocus pallasii* Goldb. subsp. pallasii Goldb. taksonları ekstraklarının aktif bileşenleri ve bazı biyolojik aktivitelerinin belirlenmesi. Yüksek Lisans Tezi, Pamukkale Üniversitesi, Fen Bilimleri Enstitüsü, Denizli.

Deveci, E., Tel-Çayan, G., Duru, M. E. (2018). Phenolic profile, antioxidant, anticholinesterase, and anti-tyrosinase activities of the various extracts of Ferula elaeochytris and Sideritis stricta. International journal of food properties, 21(1), 771-783.

Erenler, R., Genç, N, Elmastaş, M., Eminağaoğlu, Ö. (2019). Evaluation of antioxidant capacity with total phenolic content of *Galanthus krasnovii* (Amaryllidaceae). The Turkish Journal of Biodiversity, 2(1), 13-17.

Fernández-López, J., Botella-Martínez, C., Navarro-Rodríguez de Vera, C., Sayas-Barberá, M. E., Viuda-Martos, M., Sánchez-Zapata, E., Pérez-Álvarez, J. A. (2020). Vegetable soups and creams: Raw materials, processing, health benefits, and innovation trends. Plants, 9(12), 1769

Ghane, S. G., Attar, U. A., Yadav, P. B., Lekhak, M. M. (2018). Antioxidant, anti-diabetic, acetylcholinesterase inhibitory potential and estimation of alkaloids (lycorine and galanthamine) from Crinum species: An important source of anticancer and anti-Alzheimer drug. Industrial Crops and Products, 125, 168-177.

Kalayu, G., 2019. Phosphate solubilizing microorganisms: promising approach as biofertilizers. Int. J. Agron. 2019, 1–7. https://doi.org/10.1155/2019/4917256

Kang, E.L., Biscaro, B., Piazza, F., Tesco, G., (2012). BACE1 protein endocytosis and trafficking are differentially regulated by ubiquitination at lysine 501 and the Di- leucine motif in the carboxyl terminus. J. Biol. Chem. 287 (51), 42867–42880. https://doi.org/10.1074/jbc.M112.407072

Korcan, S. E., Bulduk, İ., Kahraman, T., Kayhan, R., Çitekçi, K., Kölemek., H., Öztürk, M. (2018). *Narcissus papyraceus* Soğanlarında Toplam Fenolik Bileşikler, Toplam Antioksidan Kapasite, Toplam Flavonoid Maddelerin ve Antimikrobiyal Aktivitenin Belirlenmesi. Uşak Üniversitesi Fen ve DoğaBilimleri Dergisi, 2018(1), 10-17.

Orme˜no, E., Fernandez, C., 2012. Effect of soil nutrient on production and diversity of volatile terpenoids from plants. Curr. Bioact. Compd. 8 (1), 71–79. https://doi.org/10.2174/157340712799828188

Taiz L. and E. Zeiger. 2010. Plant Physiology fifth Edition. Sinauer Associates., Inc. 23 Plum tree Road, Sunderland, MA 01375, USA

Prakash D., Singh B.N., Upadhyay G., (2007). Antioxidant and free radical scavenging activities of phenols from onion (*Allium cepa*). Food Chemistry, 102, 1389–1393.Belirlenmesi. Uşak Üniversitesi Fen ve Doğa Bilimleri Dergisi, 2018(1), 10-17.

Pesaresi, A., Lamba, D., Vezenkov, L., Tsekova, D., Lozanov, V., (2022). Kinetic and structural studies on the inhibition of acetylcholinesterase and butyrylcholinesterase by a series of multitarget-directed galantamine-peptide derivatives. Chem. Biol. Interact. 365, 110092 https://doi.org/10.1016/j.cbi.2022.110092

Semerdjieva, I., Sidjimova, B., Yankova-Tsvetkova, E., Kostova, M., Zheljazkov, V.D., (2019). Study on *Galanthus* species in the Bulgarian flora. Heliyon 5, e03021. https://doi.org/10.1016/j.heliyon.2019

Turan, M. (2016). Cyclamen alpinum ve Cyclamen parviflorum ekstraktlarının fenolik bileşenleri ve bazı biyolojik özelliklerinin belirlenmesi. Yüksek Lisans Tezi, Pamukkale Üniversitesi, Fen Bilimleri Enstitüsü, Denizli.