Original Article

Assessment of Antioxidant Activity of Giant Snowdrop (*Galanthus elwesii* Hook) Extracts with Their Total Phenol and Flavonoid Contents

Ebru Bati Ay[1*], Melek Gül[2], M. Akif Açikgöz[3], Tarik Yarilgaç[3], Ş. Metin Kara[3]

[1]Suluova Vocational School, Amasya University, Amasya, TURKEY.
[2]Faculty of Science and Art, Amasya University, Amasya, TURKEY.
[3]Faculty of Agriculture, Ordu University, Ordu, TURKEY.

ABSTRACT

Background: The aim of this study was to determine total phenolic and flavonoid contents and antioxidant activities of Giant Snowdrop (*Galanthus elwesii* Hook). **Objective:** Giant Snowdrop, the second most common snowdrop in cultivation, has a long traditional use in folk medicines as it contains some alkaloids and phenolics. **Material and Methods:** The plant material was grown in Amasya province of Turkey during autumn-winter growing period in 2016-2017. The plant samples were taken from different organs (root, leaf, flower and bulb) at different growing stages (at the beginning of flowering, after flowering and fruit ripening) and the samples were air dried at room temperature. The phenolic contents were determined by reversed phase HPLC and antioxidant properties were evaluated by free radical scavenging activity, reducing power and metal chelating capacity. **Result:** The HPLC chromatogram showed the presence of gallic acid, caffeic acid, camphorol, quercetin, myricetin and formonenitin. Higher concentrations of phenolic compounds were detected in the leaf samples than the other plant parts, especially after flowering stage. **Conclusion:** Our results showed that antioxidant activity of snowdrop might be considerably vary based on plant part and growing stage with the leaf showing higher antioxidant activity than the other plant parts.

Key words: Galanthamine, Gallic acid, HPLC, Phenolic compounds.

INTRODUCTION

The genus of *Galanthus* of the family Amaryllidaceae, consisted of approximately 1100 species in 85 genera, is well known for its alkaloids with diverse chemical structures and biological activities. *Galanthus elwesii* Hook (Giant snowdrop) is native of Asia Minor and locally distributed in the North and South Anatolian mountainous districts. *Galanthus* contains a variety of seconder metabolites, flavonoids, phenolics, terpenoids and some important alkaloids such as galanthamine and lycorine which are important for the modern pharmacological and therapeutical strategies.

Amaryllidaceae family has been proven to have a wide spectrum of biological activities including antiviral, antitumor, antibacterial and anti-inflammatory activities.[1,2] Due to their important medicinal properties, it has been of considerable interest to determine the content of the alkaloids present in the plants of Amaryllidaceae family. On the other hand, antioxidant capacity and its physiological background is poorly studied in Amaryllidaceae, even though many species of this family are known for their medicinal value.

Antioxidant activity of plants may be due to their phenolic compounds. Flavonoids are a group of polyphenolic compounds with known properties including free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action.[3] A study in *Galanthus woronowii*

Submission Date: 30-08-2017;
Revision Date: 17-11-2017;
Accepted Date: 23-11-2017

DOI: 10.5530/ijper.52.4s.88

Correspondence:
Ebru Bati Ay,
Suluova Vocational School, Amasya University, Amasya, TURKEY.
Phone: +90 535 973 0923
E-mail: ebru.bati@amasya.edu.tr

www.ijper.org

revealed that total phenolic content and consequently antioxidant activity were high.[4] In the literature, there was no study aimed at determining antioxidant activity, total phenolic and flavonoid content of giant snowdrop. Therefore, the present study was carried to determine total phenolic and flavonoid contents and antioxidant activities of giant snowdrop in terms of plant organ and growing stage.

MATERIAL AND METHOD

Plant material

Plant material was grown in Amasya province of Turkey, in autumn-winter growing period of 2016-2017. Samples were taken from different plant organs (root, leaf, flower and bulb) at different growing stages (start and after flowering, fruit ripening).

Total phenolic and total flavonoid content

The total phenolic content of the plant extracts was determined using the Folin-Ciocalteu reagent.[5] Absorbance was measured at 760 nm and standard gallic acid solution (5–1000 mg, 0.1 ml^{-1}) standard curve was obtained and the results were expressed in gram gallic acid equivalent GA/100g dry plant, which was performed duplicate. The total flavonoid content analysis of the plant extracts was determined using aluminum chloride ($AlCl_3$) and quercetin as standards.[6] The results were expressed as mg quercetin (QE/g) after obtaining the standard curve (5–1000 mg, 0.1 ml^{-1}).

Extraction procedure and determination of phenolic profile via RP-HPLC

The homogenized sample was weighed and transferred to a beaker. Then, 70% (v/v) ethanol was added into the ultrasonic bath for 3h since temperature was controlled by ice bath system setup at 30-35°C and this was produced in duplicates, The extract was then separated from the residue by filtration. The residual solvent was removed under reduced pressure. The same extraction procedure using 70% (v/v) ethanol as an extraction medium was carried out on all sample to determine antioxidant activity and phenolic content. RP-HPLC analysis of extracts was carried out using the Shimadzu Prominence Modular LC20A system via C18 column 250x4.6 mm id; 5 µm. The column temperature was controlled at 27°C and injection volume was kept 50 µL. The mobile phases were consisted of 1% acetic acid in ultrapure water (A), and methanol (B) with flow rate 0.9 ml/min. The gradient system was performed; the eluent was held at 3 min 90% A, 15 min 80% A, 12 min 75% and finally was achieved with 90 % A and 10 % B for 15 min. The quantification was made by UV-vis absorbance photodiode-array detector (DAD) at 280 nm and 320 nm. Each compound was identified by comparisons with related standards and calibration curve.

The antioxidant activity methods

Free radical scavenging activities of the plant extracts and synthetic antioxidant substances used in the study, prepared in methanol at concentrations of 25- 400 µg/mL, were determined in accordance with the Shimada method[7] based on the principle of scavenging the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical. Metal chelating activity was measured chelating of ferrous ions by extracts and standard molecules was generated via the Dinis *et al.* method.[8] The different concentrations of the extracts and standards (25–50-100-200-400 µg/mL) were reacted with $FeCl_2$ and ferrozine after finished procedure; absorbance of the solution was measured at 562 nm in multiplayer reader spectrometer. Total reducing activity was detailed the Oyaizu methods.[9] The range of 25 to 400 µg/mL concentrations of extracts were used with potassium ferric cyanide method, and the absorbance was measured at 700 nm in a spectrophotometer. Increased absorbance of the reaction mixture indicates an increase of reduction capability.

RESULTS AND DISCUSSION

The selection of objects aimed evaluating the antioxidant activities of extracts associated with flavonoid and phenolic content. Some of species with biological activity were analyzed narrow distributions.[1]

In *Galanthus elwesii* extracts, the highest phenolic content (42.63 mg of GA/g) was detected in the bulb at fruit ripening stage while the lowest phenolic content (18.15 mg of GA/g) (Table 1a) was detected in the root at fruit ripening stage. Similarly, the highest flavonoid content (58.63 mg of QE/g) was detected in the bulb at fruit ripening stage while the lowest flavonoid content (19.46 mg of QE/g) was detected in the root at fruit ripening stage (Table 1b).

Three subclasses of phenolic compounds were detected in all extracts by using HPLC, hydroxybenzoic acid and hydroxycinnamic acid, flavonol and isoflavonol. A total of the 6 phenolic compounds were identified in all growing stages (at the beginning of flowering, after flowering and fruit ripening). Higher concentrations of flavonoid and phenolic compounds were detected in the leaf samples than in the other plant parts, especially after flowering stage (Table 2).

The results of antioxidant properties of extracts estimated by the DPPH radical scavenging, metal chelating and reducing activity. All antioxidant methods were

Table 1 (a): Total phenolic content (mg of GA/g) in giant snowdrop samples taken from different plant organs at different growth stages

Plant Parts	Growing Stages		
	Beginning of flowering	After flowering	Fruit ripening
Leaf	25.19 (1)	30.18 (2)	42.58 (3)
Bulb	28.52 (4)	33.75 (5)	42.63 (6)
Root	22.25 (7)	20.18 (8)	18.15 (9)
Flower	19.58 (10)		

Table 1 (b): Total flavonoid content (mg of QE/g) in giant snowdrop samples taken from different plant organs at different growth stages

Growing Stages		
Beginning of flowering	After flowering	Fruit ripening
29.26	32.57	43.21
27.63	32.47	58.63
21.56	21.22	19.46
28.05		

Table 2: Quantitation of phenolics of giant snowdrop via RP-HPLC (DAD).

Alkaloids	Growing Stages								
	Beginning Flowering			After Flowering			Fruit Ripening		
	Plant Parts			Plant Parts			Plant Parts		
	Leaf	Bulb	Root	Leaf	Bulb	Root	Leaf	Bulb	Root
Gallic acid	18.70	7.46	5.53	24.10	10.48	5.73	14.11	12.41	15.03
Caffeic acid	10.12	5.82	ND	13.26	1.78	10.91	8.04	ND	ND
Myrecetin	20.83	2.80	ND	7.09	ND	1.41	3.6	1.53	ND
Kaemferol	12.18	1.62	1.91	6.81	ND	1.05	5.38	ND	ND
Formononetin	12.62	ND	1.71	10.73	ND	2.14	5.29	ND	ND
Quercetin	27.69	9.3	5.48	28.7	4.6	8.76	18.3	8.78	10.93

ND: Not detected

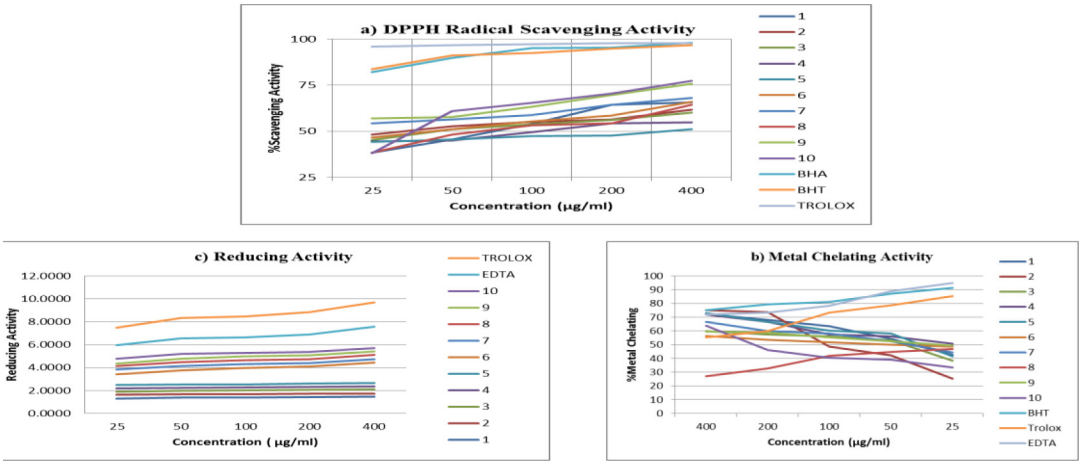


Figure 1: Antioxidant activity of giant snowdrop samples taken from different plant parts at different growth stages

significantly affected by growing stages and plant organs with interaction between the two (Figure 1). The same type of correlation between antioxidant activity and phenolic content has been demonstrated in different growing stage as well as their plant part. Under 3 methods of antioxidative activities results utilized in the work showed parallel sequence of activity: Fruit ripening (3,6,9) > After flowering (2,5,8) > Beginning of flowering (1,4,7). Remarkable differences among leaf, bulb, root, and flower were found for the target antioxidative activity: leaf > flower > bulb > root.

CONCLUSION

Higher concentrations of flavonoid and phenolic compounds were detected in the leaf samples than in the other plant parts of giant snowdrop, especially after flowering stage. Our results showed that antioxidant activity of snowdrop might vary based on plant organs and plant growing stages, with the leaf showing higher antioxidant activity than the other plant parts.

ACKNOWLEDGMENT

Scientific Research Projects Unit (BAP) of Ordu University for providing support to this research, as a part of TF-1645 BAP Project. The authors wish to thank to the members of Research Central Laboratory of Amasya University for their help in HPLC analysis.

CONFLICT OF INTEREST

The authors have no conflict of interest.

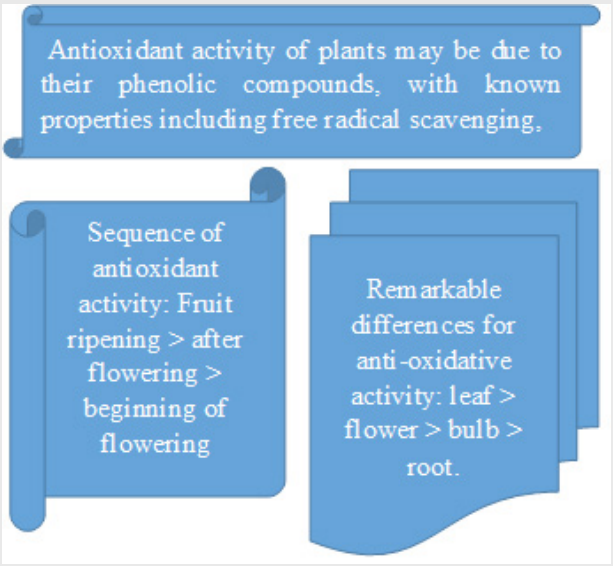
ABBREVIATIONS

GA: Gallic Acid; **QE:** Quercetin; **DPPH:** Free radical scavenning activity; **HPLC:** High performance liquid chromatography.

REFERENCES

1. Jessamyn RL, Little JR, Brennan D, Bastida J. Structure–activity studies on acetylcholinesterase inhibition in the lycorine series of Amaryllidaceae alkaloids. Bioorganic and Medicinal Chemistry Letters. 2010;20(17):5290-4.
2. Powell KS, Spence J, Bharathi M, Gatehouse JA, Gatehouse AM. Immune histochemical and developmental studies to elucidate the mechanism of action of the snowdrop lectin on the rice brown plant hopper, Nilaparvata lugens (Stal). Journal of Insect Physiology. 1998;44(7):529-39.
3. Balasundram N, Sundram K, Samman S. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food chemistry. 2006;99(1):191-203.
4. Resetár A, Freytag C, Kalydi F, Gonda S, M-Hamvas M, Ajtay K, *et al.* Production and antioxidant capacity of tissue cultures from four Amaryllidaceae species. Acta Soc Bot Pol. 2017; 86(1):3525.
5. Wolfe K, Wu X, Liu RH. *Antioxidant Activity* of Apple Peels. J. Agric. Food Chem. 2003;51: 609-14.
6. Park YS, Kim SJ, Chang HI. Isolation of Anthocyanin from Black Rice (Heugjinjubyeo) and Screening of its Antioxidant Activities. Kor. J. Microbiol. Biotechnol. 2008;36(1):55-60.
7. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthone on the auto oxidation of soybean in cylcodextrin emulsion. J. Agr. Food Chem. 1992;40(6):945-8.
8. Dinis TCP, Madeira VMC, Almeida LM. Action of phenolic derivatives (aceto aminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys. 1994;315:161-9.
9. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. Japanese Journal of Nutrition. 1986;44(6):307-15.

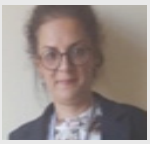
PICTORIAL ABSTRACT



SUMMARY

- Giant Snowdrop, including certain alkaloids and phenolic compounds, shows antioxidant activity and has a long traditional use in folk medicine.
- The samples taken from different organs (root, leaf, flower, and bulb) at three growth stages (beginning and after flowering, fruit ripening) were used to monitor alkaloid and phenolic contents and antioxidant properties.
- Higher concentrations of phenolic compounds were detected in the leaf samples, particularly after flowering, with higher antioxidant activity.
- Antioxidant activity of snowdrop may show a considerable variability based on plant parts and growing stages.

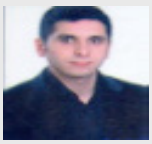
About Authors



Ebru Batı Ay: She is the teaching and research instructor at Medicinal and Aromatic Plants program, Suluova Vocational School of Amasya University. She is studying for her doctorate degree at the Department of Field Crops of Agricultural Faculty, Ordu University. She mainly focuses on medicinal and aromatic plants and secondary metabolites.

Melek Gül: She attended the University of Amasya. She received her Ph.D.in chemistry in 2009 from Y□ldız Technical University working with Prof. Nuket Öcal, and was a postdoctoral research at Ioannina University with Prof. Yiannis Elemes. Assoc.Prof. Gül's research interests include organic synthesis and medicinal chemistry.

Prof. Dr. Şevket Metin Kara: He is the professor at the Department of Field Crops of Agricultural Faculty, Ordu University. His area of research is oil crops and medicinal and aromatic plants. He has more than 20 years of teaching and research experience in Field Crops.



Muhammed Akif Açıkgöz: He is the doctor of Department of Field Crops of Agricultural Faculty, Ordu University of Turkey. His research areas include plant biotechnology, tissue culture, volatile oils and secondary metabolites in medicinal and aromatic plants.

Cite this article: Bati Ay E, Gül M, Açikgöz MA, Yarilgaç T, Kara SM. Assessment of Antioxidant Activity of Giant Snowdrop (*Galanthus elwesii* Hook) Extracts with Their Total Phenol and Flavonoid Contents. Indian J of Pharmaceutical Education and Research. 2018;52(4S):S128-S132.