PAPER DETAILS

TITLE: Evaluation of antioxidant capacity with total phenolic content of Galanthus krasnovii (Amaryllidaceae)

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PAGES: 13-17

ORIGINAL PDF URL: http://turkbiod.artvin.edu.tr/tr/download/article-file/684848

Turkish Journal of Biodiversity

Turk J Biod, March 2019, 2(1): 13-17
Journal homepage: http://turkbiod.artvin.edu.tr/
http://dergipark.gov.tr/biodiversity

e-ISSN:2667-4386

RESEARCH ARTICLE Open Access

Evaluation of antioxidant capacity with total phenolic content of *Galanthus krasnovii* (Amaryllidaceae)

Galanthus krasnovii (Amaryllidaceae)'nin toplam fenolik içeriği ile antioksidan kapasitenin değerlendirilmesi

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Article Info

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Article history
Received: February 13, 2019
Received in revised form: March 12, 2019
Accepted: March 13, 2019
Available online: March 15, 2019

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Keywords:
Galanthus krasnovii, antioxidant, natural product, phenolic contents.

Anahtar kelimeler:
Galanthus krasnovii, antioksidan, doğal ürün, fenolik içerik.

ABSTRACT

Natural products have gained the great interest due to their broad spectrum of biological activities. *Galanthus krasnovii* was dried at shade then extracted with hexane, dichloromethane, and ethyl acetate successively. After removing of solvent by reduced pressure, crude extracts of each solvent were yielded. Antioxidant activity including 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical cation and reducing power assays were carried out for each extract. Moreover, total phenolic content was determined. Total phenolic content of hexane-, dichloromethane-, and ethyl acetate extracts were found as 60.95 mg GAE/g extract, 71.90 GAE/g extract and 58.90 GAE/g extract respectively. Dichloromethane and ethyl acetate extract revealed the moderate antioxidant activity.

ÖZ

Doğal ürünler geniş spektrumlu biyolojik aktivite göstermelerinden dolayı yoğun ilgi görmektedirler. *Galanthus krasnovii* gölgede kurutulduktan sonra sırasıyla hekzan, diklorometan ve etil asetat ile ekstrakte edildi. Düşük basınçta çözücü uzaklaştırıldıktan sonra her bir çözücünün ekstraktı elde edildi. Her bir ekstraktın, 1,1-difenil-2- pikralhidrazil(DPPH) radikal, 2,2'-azino-bis(3- etilbenzotiazolin-6-sülfirik asit) (ABTS) radikal katyon ve indirgeme gücü antioksidan çalışmaları gerçekleştirildi. Ayrıca, ekstraktların toplam fenolik içerikleri belirlendi. Hekzan-, diklorometan- ve etil asetat ekstraktlarının toplam fenolik içerikleri sırasıyla 60.95 mg GAE/g ekstrakt, 71.90 GAE/g ekstrakt ve 58.90 GAE/g ekstrakt olarak belirlendi. Diklorometan ve etil asetat ekstraktları orta derece aktivite gösterdi.

Citation:

To cite this article: Erenler R, Genç N, Elmastaş M, Eminağaoğlu Ö (2019). Evaluation of antioxidant capacity with total phenolic content of *Galanthus krasnovii* (Amaryllidaceae). Turk J Biod 2(1): 13-17.

1. INTRODUCTION

Plants play a significant role in drug discovery process. Since they reveal plenty of pharmaceutical properties, isolation of bioactive compounds from plants gains the great interest to find the most effective compounds with activity mechanism (Aksit et al., 2014; Demirtas et al., 2013; Elmastas et al., 2016; Elmastaş et al., 2015; Erenler, Demirtas, et al., 2017; Erenler, Meral, et al., 2017; Erenler, Sen, Aksit, et al., 2016; Erenler, Sen, Yaglioglu, et al., 2016; Erenler et al., 2018; Guzel et al., 2017; Karan et al., 2017; Karan & Erenler 2017a; Karan & Erenler 2018; Karan & Erenler 2017b; Karan et al., 2018; Topçu et al., 1999; Yaglıoglu et al., 2013; Yildiz et al., 2017).

Galanthus L. genus, known as snowdrops, belonging to Amaryllidaceae family consists of 20 species distributed throughout Europe, Asia and Near East (Zubov & Davis 2012). Six species are endemic for Turkey flora (Davis & Özhatay, 2001). Turkey is one of the centers of species

diversity of *Galanthus*. It is the most traded ornamental bulb genus in the world and a great number of bulbs are exported from Turkey each year. This genus is very popular garden plant in Europe and is especially considered as the early messenger of spring. Some of the *Galanthus* species have been included in the red list of threatened species (Convention on International Trade in Endangered Species of Wild Fauna and Flora – CITES) due to the extensive collection, climate change, and habitat destruction.

Previous phytochemical study on *Galanthus* species provided the isolation of bioactive alkaloids such as tazettine, galanthusine, molycorine, galanthamine, gananthine (Berkov et al., 2012; Kintsurashvili & Vachnadze, 2007). *Galanthus woronowii* Losinsk. collected from Trabzon, Turkey included the galanthine, galanthamine, 2-*O*-(3'-hydroxybutanoyl) lycorine, narwedine, *O*-methylleucotamine, sternbergine, lycorine, sanguinine, salsoline (Sarikaya et al., 2013). *Galanthus* species have been used in homeopathy to treat various illness such as headaches, cardiac failure, mitral disorder (Bokov & Samylina, 2016). Galanthamine, a main constituent of *Galanthus* species, is a licensed drug for the treatment of dementia in Alzheimer's disease. It inhibits the Acetylcholinesterase (AChE) selectively and competitively (Ellis et al., 2009).

Reactive oxygen species are free radicals yielding in human body during the oxidative function. Human body has defense mechanism against oxidative stress such as enzymes and compounds. Natural antioxidants become inadequate under some circumstance such as malnutrition, ultra violet radiation, and smoking. Hence, excess free radical is able to damage to cell membrane leading to degenerative illness and conditions such as Alzheimer's disease, cardiovascular disease, process, diabetes, inflammation and DNA injury leading to carcinogenesis. Antioxidants play an important role for inhibiting or quenching free radicals. Antioxidants have been used in food, cosmetics and medicine to replace synthetic antioxidants forbidden because of their carcinogenicity (Sasaki et al., 2002). There is not any report about the antioxidant activity of *Galanthus krasnovii* A.P.Khokhr.

In this work, it was presented the antioxidant potency of *Galanthus krasnovii* using $DPPH^{\bullet}$, $ABTS^{\bullet+}$, and reducing power assays. In addition, total phenolic content was executed.

2. MATERIAL AND METHOD

2.1. General experimental procedures

UV analysis was carried out with Hitachi U-290 UV–VIS spectrophotometer. Potassium persulphate ($K_2S_2O_8$), trolox, amonyum acetate ($NH_4CO_2CH_3$), butlylaed hydroxytoluene (BHT), butlylated hydroxyanisole (BHA), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH) were supplied by Sigma (Sigma-Aldrich GmbH, Steinheim, Germany). The solvents with analytical grade, potassium dihydrogen phosphate (KH_2PO_4), potassium hydroxide (KOH), cupper (II) chloride ($CuCl_2$), Folin-Ciocalteu reagent, gallic acid were bought from E. Merck (Darmstadt, Germany).

2.2. Plant material

The voucher specimens have been deposited at the Herbarium of Artvin Coruh University (ARTH), Artvin, Turkey. *G. krasnovii* was collected from Turkey: A8 Artvin, Kafkasör, 1100 m, in 05.04.2018 and identified by Prof. Dr. Özgür Eminağaoğlu, specialists of Plant Taxonomy (ARTH 13437).

2.3. Extraction

G. krasnovii (100 g) was extracted with hexane (2 × 100 mL), dichloromethane (2 × 100 mL) and ethyl acetate (2 × 100 mL) sequentially for 2 days. After removing of solvent from each extract solution under reduced pressure, the crude extracts of hexane (1.5 g), dichloromethane (1.2 g) and ethyl acetate (1.3 g) were yielded. The extracts were kept in fridge to analyze the antioxidant activity with total phenolic content (Genç et al., 2019).

2.4. Total phenolic determination

Total phenolic compounds of *G. krasnovii* extracts (hexane, dichloromethane and ethyl acetate) was executed by Folin-Ciocalteu method (Singleton & Slinkard 1977). The extract solution (1.0 mg/1mL) was reacted with Folin ciocalteu (0.1 mL) in distillated water (4.6 mL). After addition of sodium carbonate solution (0.3 mL, 2%), the reaction mixture was incubated for 2 h at room temperature. The absorbance was determined at 765 nm by a spectrophotometer. The standard curve was calculated using gallic acid and the results were presented

as gallic acid equivalents per mg of extract (Erenler et al., 2014). All experiments were carried out in triplicate.

2.5. DPPH free radical scavenging assay

DPPH$^{\bullet}$ activity of hexane-, dichloromethane- and ethyl acetate extracts of *G. krasnovii* was carried out by the protocol reported (Blois 1958). DPPH$^{\bullet}$ (1.0 mL, 0.26 mM) was reacted with various concentration of each extract solution (3.0 mL) for 15 min at rt. Tha absorbance was determined at 517 nm. BHA, BHT and Trolox were used as standard controls. IC_{50} values indicate the concentration of sample that scavenges 50% of DPPH free radical. The DPPH$^{\bullet}$ scavenging activity was calculated using the equation:

DPPH$^{\bullet}$ scavenging effect (%) = $[(A_1 - A_2) / A_1] \times 100$

A_1 is the absorbance of the control and A_2 is the absorbance of the sample (Elmastas et al. 2004).

2.6. ABTS radical cation scavenging assay

ABTS radical cation activity was executed by the protocol reported previously (Re et al. 1999). Initially, ABTS$^{\bullet+}$ stock solution was prepared by the treatment of ABTS (2 mM) with potassium persulfate (2.45 mM). Later, it was kept for 6 h in dark at rt. Each extract solution (3.0 mL) was reacted with ABTS$^{\bullet+}$ solution (1.0 mL). The absorbance was determined at 734 nm by a spectrophotometer. The inhibition was calculated for each concentration in comparison to a blank absorbance (Erenler et al. 2015). ABTS$^{\bullet+}$ activity was calculated by the equation: ABTS$^{\bullet+}$ scavenging effect (%) = $[(A_1 - A_2) / A_1] \times 100$ in which, A_1 is ABTS$^{\bullet+}$ initial concentration and A_2 is ABTS$^{\bullet+}$ remaining concentration in the sample. The results were calculated as IC_{50}.

2.7. Cupric ion reducing power assay

Cupric ion reducing power assay was carried out for each extract of *G. krasnovii* (Elmastas et al., 2018). The reduction capacity of each extract can be calculated by the efficient reduction of Cu^{+2} to Cu^{+1}. The yellow complex formed after addition of cupper (I) chloride. The extract (40-160 µg/mL, 1.0 mL), $CuCl_2$ (0.01 M, 1.0 mL), neocuproine (1.0 mL, 7.5×10^{-3} M), and acetate tampon (1.0 mL, 1.0 M) were added to the reaction flask and incubated for 30 min. The absorbance was recorded at 450 nm (Apak et al., 2004). The result was expressed according to the Trolox equivalent (µmol/g sample).

3. RESULTS AND DISCUSSION

Galanthus species are significant plants including the bioactive compound used in drug. Some species of *Galanthus* genus were reported to reveal the antioxidant activity. Antioxidant activity of various parts (root, leaf, flower and bulb) of *Galanthus elwesii* Hook.f. was executed at different growing stages such as beginning, flowering, after flowering and fruit ripening. The part of the plant and growing stage altered the activity significantly. The leaf at fruit ripening stage of *G. elwesii* displayed the most antioxidant activity (Ay et al., 2018). *G. transcaucasicus* Fomin shoot extract revealed the considerable antioxidant and antimicrobial activity (Karimi et al., 2018). In this study, hexane-, dichloromethane- and ethyl acetate extracts of *Galanthus krasnovii* were investigated for antioxidant activity as well as total phenolic content. Total phenolic content of hexane, dichloromethane and ethyl acetate extracts was found as 60.95 mg GAE/g extract, 71.90 GAE/g extract and 58.90 GAE/g extract respectively. Hexane extract did not reveal the DPPH free radical activity. However, dichloromethane extract and EtOAc extract exhibited the moderate activity (Figure 1).

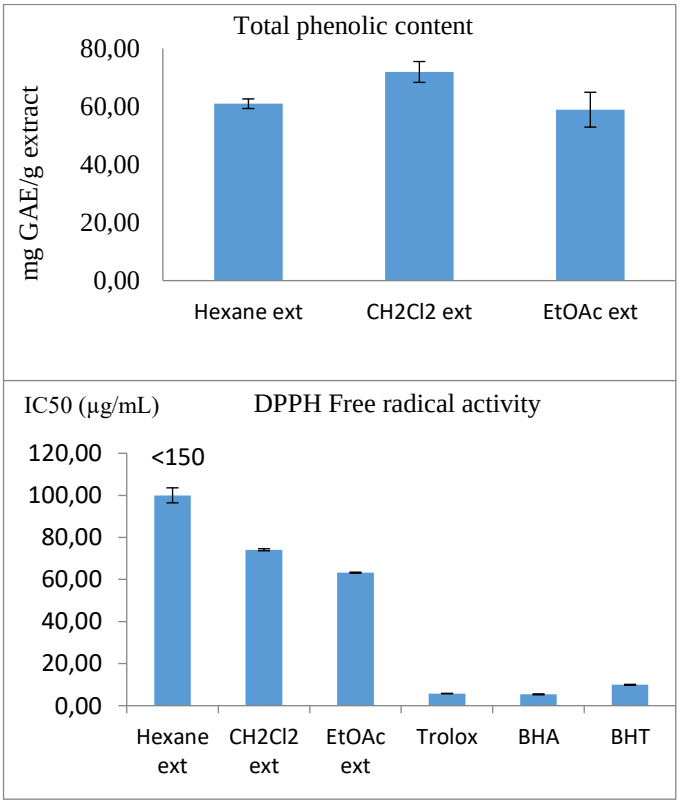

Figure 1. Total phenolic content and DPPH free radical activity

In regarding ABTS radical cation activity, dichloromethane extract and EtOAc extract displayed the mild activity with 14.33 (IC_{50}, µg/mL) and 14. 98 (IC_{50}, µg/mL) respectively compared to the standard BHA (8.8, IC_{50}, µg/mL). Reducing power activity was executed with respect to trolox equivalent. Dichloromethane extract (1.15 µmol TE / mg extract) displayed the most reducing power effect among the extracts tested. Ethyl acetate and hexane extracts revealed the activity of 0.77 µmol TE / mg extract and 0.75 µmol TE / mg extract, respectively. This result revealed that the compounds in dichloromethane extract had electron donation ability to reduce from Cu^{+2} to Cu^{+1}.

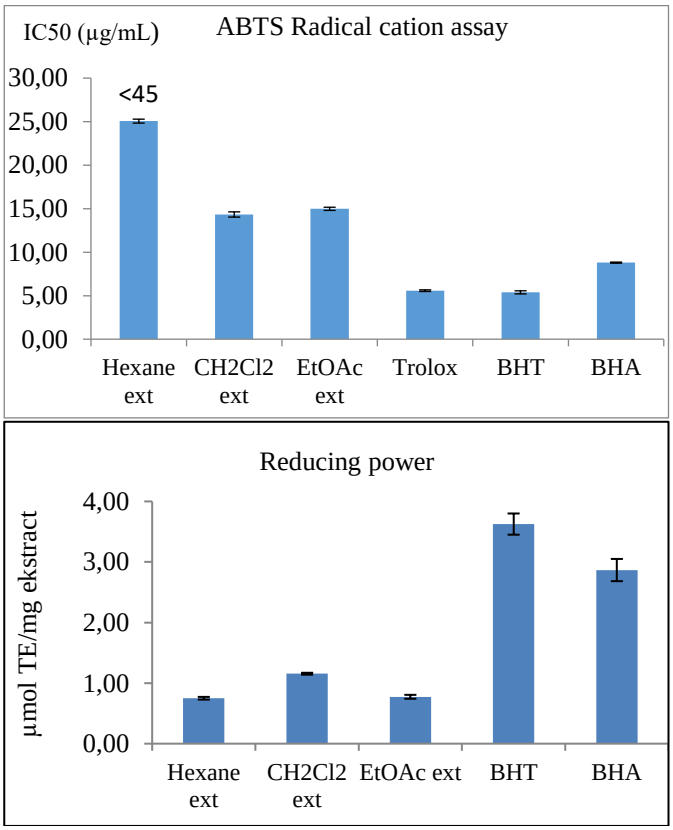

Figure 2. ABTS Radical cation assay and reducing power

4. CONCLUSIONS

G. krasnovii has a potency to be used in pharmaceutical and food industry as a natural antioxidant. The cultivation area of *G. krasnovii* should be enlarged. Bioactive compounds should be isolated and alkaloids quantity of *G. krasnovii* should be increased by using various agricultural techniques. Antioxidant effects of isolated compounds should be investigated and activity-structure mechanism should be presented.

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