KMÜ Mühendislik ve Doğa Bilimleri Dergisi
https://dergipark.org.tr/tr/pub/kmujens
5(2), 118-130, (2023) © KMUJENS
e-ISSN: 2687-5071
https://doi.org/10.55213/kmujens.1374619

Determination of Free Radical Scavenging and Metal Chelating Activities of Different Extracts of *Galanthus elwesii* Hook.f.

Galanthus elwesii Hook.f.'nin Farklı Ekstraktlarının Serbest Radikal Yakalama ve Metal Şelatlama Aktivitelerinin Tespit Edilmesi

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(Received: 11 October 2023; Accepted: 31 October 2023)

Abstract. Among antioxidants effective in combating oxidative stress, natural compounds play a significant role. Many plants may have high levels of antioxidant capacity through the compounds they contain. In line with this information, in the current study, we aimed to evaluate the antioxidant properties of *Galanthus elwesii* Hook.f. In this context, it was tested the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging and metal chelating activities of different concentrations (12.5-400 mg/L) of methanol and water extracts obtained from the bulb part of this plant. A concentration-dependent increase in activity was observed for both extracts. DPPH scavenging (87.10%) and metal chelating (72.99%) activities demonstrated by 400 mg/L concentration applications came to the fore. In addition, it was determined that these data were significantly ($p < 0.05$) higher than the activity rates revealed by other applications. Considering the IC_{50} values, low values for DPPH scavenging and metal chelating activities (110.88 mg/L and 132.40 mg/L, respectively) were detected in

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water extracts. As a result, the importance of *G. elwesii* among plants that have the potential to be a rich source of antioxidants was revealed in this study.

Key words: Antioxidant, herbal product, natural compound

Özet. Oksidatif stresle mücadelede etkili olan antioksidanlar arasında, doğal bileşikler önemli bir rol oynamaktadır. Birçok bitki taşıdıkları bileşikler aracılığı ile yüksek seviyede antioksidan kapasiteye sahip olabilir. Bu bilgiler doğrultusunda, mevcut çalışmada, *Galanthus elwesii* Hook.f.'nin antioksidan özelliklerini değerlendirmeyi amaçladık. Bu kapsamda, bu bitkinin soğan kısmından elde edilen metanol ve su ekstraktlarının farklı konsantrasyonlarının (12,5-400 mg/L) 1,1-difenil-2-pikrilhidrazil (DPPH) yakalama ve metal şelatlama aktiviteleri test edildi. Her iki ekstrakt için de konsantrasyona bağlı aktivite artışı görülmüştür. 400 mg/L konsantrasyonlu uygulamaların gösterdiği DPPH yakalama (%87,10) ve metal şelatlama (%72,99) aktiviteleri ön plana çıkmıştır. Ayrıca bu verilerin diğer uygulamaların ortaya çıkardığı aktivite oranlarından önemli derecede ($p < 0,05$) yüksek olduğu belirlenmiştir. IC_{50} değerleri göz önüne alındığında, DPPH yakalama ve metal şelatlama aktiviteleri için düşük değerler (sırasıyla, 110,88 mg/L ve 132,40 mg/L) su ekstraktlarında tespit edilmiştir. Sonuç olarak, antioksidanlar bakımından zengin bir kaynak olabilme potansiyeline sahip olan bitkiler içerisinde *G. elwesii*'nin önemi bu çalışma ile ortaya çıkarılmıştır.

Anahtar Kelimeler: Antioksidan, bitkisel ürün, doğal bileşik

1. Introduction

Antioxidants are molecules or compounds that neutralize or neutralize harmful free radicals formed in chemical reactions called oxidation or oxidative stress. Oxidation is a normal metabolic process in our body and serves many important functions [29,34]. However, oxidative stress occurs when free radicals are produced uncontrollably or are not neutralized. These free radicals have the potential to damage biomolecules such as DNA, proteins, and lipids, which can lead to cell damage and various health problems [23,26].

Some metal ions can trigger free radical production or increase oxidative stress [24,27]. Some metals, especially iron and copper, have the potential to form free radicals within the cell. Metal chelates can prevent or reduce excess metal ions from forming free radicals [28,37]. Chelating metal ions can reduce the potential of these ions to cause oxidative stress. On the other hand, excessive metal chelates can also be harmful. For example, it is possible for lead to accumulate in the body and cause toxicity when combined with chelating agents [1]. Therefore, it is important to maintain the balance between metal ions and free radicals in the body. Measures such as a healthy diet, consuming foods containing antioxidants, controlled intake of metal ions and avoiding toxic exposure can reduce the negative effects of metal chelation and oxidative stress on health [14,19,38].

Antioxidants reduce the negative effects of oxidative stress by neutralizing or inactivating free radicals [17,33] and they play roles in reducing the harmful effects of free radicals, such as scavenging free radicals, protecting cell membranes, protecting DNA, and reducing inflammation [22,31].

Plants are natural sources containing a variety of antioxidants and provide important health benefits for humans [20]. Antioxidants in plants protect body cells from the harmful effects of oxidative stress by neutralizing or inactivating reactive molecules known as free radicals [35]. *Galanthus* species, especially snowdrop plants, contain some alkaloids. The most well-known of these alkaloids is called galantamine. Galantamine is similar in structure to drugs such as donepezil, a drug used to relieve symptoms of Alzheimer's disease [2,30]. Therefore, some studies have examined whether galantamine in *Galanthus* species may be a potential contribution to the treatment of different diseases. *Galanthus* species have been used in some local traditional medicine practices [15]. However, scientific evidence about how these plants are used in traditional medicine and their effects is limited [7]. In this study, we aimed to examine the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging and metal chelating activities of methanol and water extracts obtained from *Galanthus elwesii* Hook.f..

2. Materials and methods

2.1. Collection and identification of the plant samples

Galanthus elwesii samples were collected from Karadağ Mountain (Karaman/Türkiye). Plant species were identified with the help with various literatures [16,21]. Whole plant samples were dried in an oven at 40°C to a constant weight after they were cleaned from soil and waste. Later they were grinded for the study.

2.2. Preparation of the extracts

Bulb samples of *G. elwesii* were dried under room conditions and powdered with an ultra-centrifuge grinder (Retsch ZM 200, Germany). The ground-dried plant samples (1.75 g) were extracted in 250 mL of methanol and water at room temperature through the Soxhlet extraction apparatus. The crude extracts of the plant samples were filtered through the Whatman No. 1 filter paper. The solvent was evaporated with a rotary evaporator (IKA, Staufen Germany) under vacuum to dry and lyophilized to get ultra-dry powders. Methanol and water extracts of *G. elwesii* yielded 20% and 12% (w/w) of plant substances, respectively.

2.3. Free radical scavenging activity

In the measurement of DPPH scavenging activity of methanol and water extracts obtained from the plants, applications were carried out with the final concentrations of the extracts in the plate wells of 12.5, 25, 50, 100, 200, and 400 mg/L. According to the method, 20 µL of the extracts were placed in each microplate well, and 180 µL of DPPH (0.06 mM in methanol) was added. The reduction of DPPH free radical was determined by measuring the absorbance values at 517 nm after 60 minutes in the dark. The free radical scavenging activities of the extracts were calculated as a percentage using the following formula: Radical scavenging activity = [(Control absorbance – Extract absorbance) / Control absorbance)] × 100.

2.4. Metal chelating activity

In the measurement of the metal chelating activity of methanol and water extracts obtained from the plants, applications were carried out with the final concentrations of the extracts in the plate wells of 12.5, 25, 50, 100, 200, and 400 mg/L. According to the method, 50 µL of the extracts were added to each microplate well. 10 µL of ferrozine (5 mM), 5 µL of $FeCl_2$ (2 mM), and 185 µL of methanol were added to them, respectively, and kept at room temperature for 10 minutes. Spectrophotometric measurements were performed at 562 nm. The metal chelating activities of the extracts were calculated in percent with the following formula: Metal chelating activity = [(Control absorbance – Extract absorbance) / Control absorbance)] × 100.

2.5. Statistical analyses

Statistical analysis was performed for antioxidant experiments using a one-way ANOVA test (Duncan). The probit model was employed to determine the values of the median inhibitor concentration (IC_{50}). Three-dimensional (3-D) density analysis was used to determine the correlations between data points. Heatmap and cluster analyses were used to group data points that share similar characteristics or are close to each other. All of these analytical procedures were conducted using SPSS software (version 21.0, IBM Corporation, Armonk, NY, USA).

3. Results and Discussion

There was a concentration-dependent increase in activity in both methanol and water extracts in DPPH scavenging activity. The maximum concentration of water extract (400 mg/L) showed the highest activity (87.10%). This value was significantly ($p < 0.05$) higher than all other values. The highest activity in the methanol extract was 72.16% (Figure 1). Considering the IC_{50} values, it was determined that the water extract was more effective (IC_{50}: 110.88 mg/L) (Table 1). There are studies in the literature showing that different compounds of *G. elwesii* have antioxidant activity. In a study, the total phenolic content change in the plant was examined through different compounds applied to this plant. The greatest total phenolic content was found in the combination of the bulb and root of the tallest plant that had been exposed to jasmonic acid [6]. In another study, it was undertaken to examine how applying foliar salicylic acid and zinc treatments affect the levels of proline, carotenoids, and chlorophyll, as well as the activity of antioxidant enzymes in *G. elwesii*. The research revealed that higher doses of salicylic acid and zinc led to an increase in malondialdehyde content, although this increase was not statistically significant. Specifically, the total chlorophyll saw an increase with Zn treatments [25]. Similarly, in a different study, the aim was to assess how the presence of zinc and phosphorus influences the production of alkaloid compounds and the bioactive properties in *G. elwesii*. An increase in antioxidant activities in the plant was observed with zinc application. Additionally, the plant extracts showed antimicrobial and cytotoxic activity [4].

A concentration-dependent increase was detected, similar to DPPH scavenging activity. When methanol and water extract were compared, it was determined that the water extract showed more effective metal chelating activity. The highest rate (72.99%) belonged to the concentration of 400 mg/L of water extract. Considering the high metal chelating activities, the concentration of 200 mg/L (57.15%) of the water extract was also noted. These two data were statistically ($p < 0.05$) different from the other data (Figure 2). When the IC_{50} values were taken into account, the water extract had a lower value (132.40 mg/L), and therefore, stood out as a more effective metal chelator (Table 1). In one of the previous studies, it was involved assessing the polyphenolic compositions of various components of *G. elwesii*, including its flowers, bulbs, and leaves, using colorimetric techniques. Furthermore, the research investigated the antioxidant attributes and the potential for inhibiting enzymes in the extracted substances. The leaf extracts of *G. elwesii* under investigation displayed the highest levels of total phenolic and total flavonoid content. All of the extracts exhibited significant activity in inhibiting acetylcholinesterase and tyrosinase [18]. Bati Ay et al. [5] assessed phenolic content using reversed-phase HPLC, while the antioxidant properties were examined through free radical scavenging capacity, reducing power, and metal chelation ability. They detected DPPH and metal chelating activities in high concentrations using concentrations similar to those used in our study. Furthermore, important natural compounds such as gallic acid, formononetin, camphorol, quercetin, caffeic acid and myricetin were determined in *G. elwesii*. The chemical composition of *G. elwesii* was studied using the GC-MS technique, and its anticholinesterase activity was examined alongside molecular modelling studies. The extracts were tested for their ability to inhibit acetylcholinesterase and butyrylcholinesterase, and many compounds were identified using GC-MS in six distinct alkaloid extracts [13]. Berkov et al. [9] also carried out a different study on the alkaloid size contents of *G. elwesii*. In different populations of *G. elwesii*, tyramine-type protoalkaloids, specifically hordenine and its variations, were found to be the prevalent compounds. There are many studies on the alkaloid content of *G. elwesii* in the literature

[8,10–12]. In addition, *G. elwesii* exhibits antioxidant activity potential due to the abundance of phenolic compounds it contains [3,32,36].

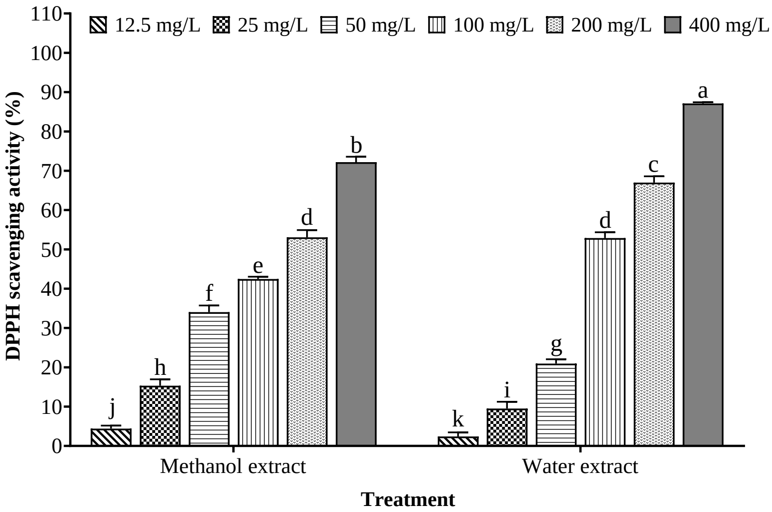

Figure 1. Evaluation of the ability of various extracts from *G. elwesii* to neutralize DPPH radicals. Vertical bars depict the standard deviation observed across five separate experiments. Dissimilar letters are used to indicate statistically significant distinctions ($p < 0.05$).

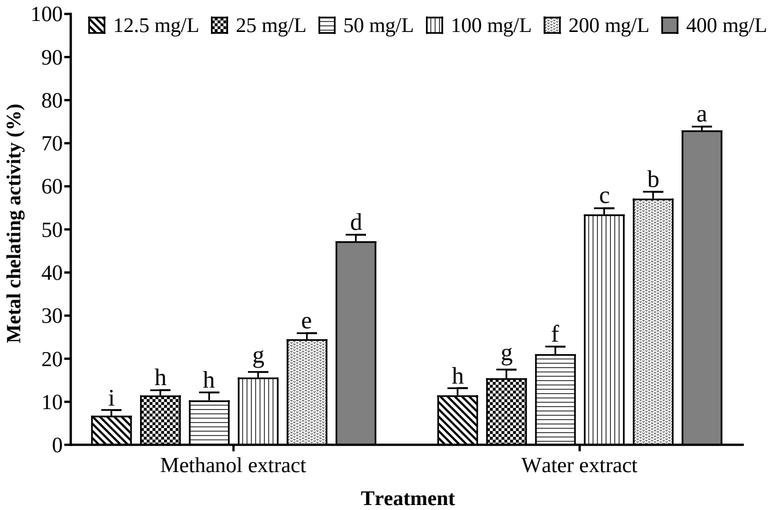

Figure 2. Evaluation of the ability of various extracts from *G. elwesii* to assessment of their capacity to chelate metals. Vertical bars depict the standard deviation observed across five separate experiments. Dissimilar letters are used to indicate statistically significant distinctions ($p < 0.05$).

Table 1. IC_{50} values (mg/L) of extracts derived from *G. elwesii* in relation to their ability to scavenge DPPH radicals and chelate ferrous ions

Extract	Activity	IC_{50} (Limits)	Slope ± Standard error (Limits)
Methanol	DPPH scavenging	144.85 (132.26–159.60)	1.34 ± 0.05 (1.23–1.44)
	Metal chelating	788.77 (610.55–1083.05)	0.91 ± 0.05 (0.80–1.02)
Water	DPPH scavenging	110.88 (104.27–118.06)	2.04 ± 0.06 (1.91–2.17)
	Metal chelating	132.40 (120.85–145.85)	1.30 ± 0.05 (1.20–1.40)

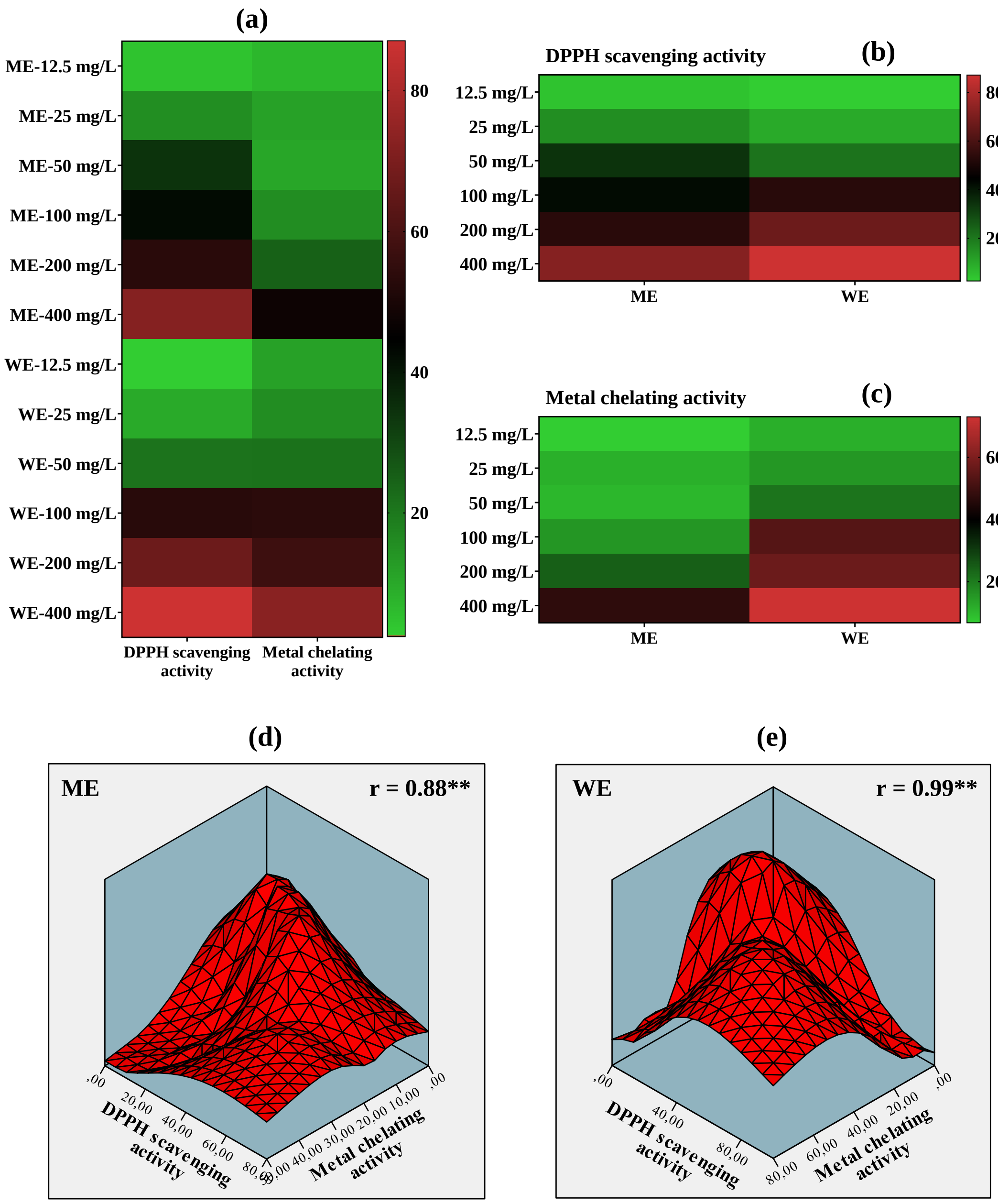

Figure 3. (a, b, c) Heatmaps depicting the percentage of DPPH scavenging and metal chelating activities of different concentrations of the extracts are presented. In the heatmap analyses, high and low activities are indicated by red and green colors, respectively. **(d, e)** Three-dimensional density analysis was conducted for DPPH scavenging and metal chelating activities of the extracts. In the 3-D density analysis, correlation coefficients (**r**) were calculated. The double asterisks (**) emphasize the significance level of 0.01. ME refers to the methanol extract of *G. elwesii*, WE refers to the water extract of *G. elwesii*.

When an examination was made between the extracts by heatmap analysis for DPPH scavenging activity, red gradients representing high activity appeared at concentration of 400 mg/L. In addition, the activity elicited by concentrations of 12.5 and 25 mg/L had a green gradient (Figure 3b). Heatmap analysis for metal chelating activity revealed low and near activities in the range of 12.5-50 mg/L with the green gradients (Figure 3c).

When DPPH scavenging and metal chelating activities were generally compared by heatmap analysis for water extract, it was determined that low (12.5-50 mg/L) and high (100-400 mg/L) concentrations showed similar activities to each other. As for the methanol extract, predominantly low concentrations (12.5 and 25 mg/L) showed similar activities (Figure 3a). In 3-D density analysis carried out to determine the correlation level between these two activities, it was revealed that the water extract showed a significantly ($p < 0.01$) high correlation ($r = 0.99$). In addition, methanol extract was also found to have a high correlation coefficient ($r = 0.88$) (Figure 3d,e).

In conclusion, the antioxidant importance of the *G. elwesii* is quite remarkable based on the results of our study. Our research has shown that this plant has high DPPH and metal chelating activities. The antioxidant compounds contained in the plant can reduce cellular damage by fighting free radicals and offer a number of health benefits. Furthermore, the antioxidant potential of *G. elwesii* indicates great potential in various application fields. Further research on this plant may provide important contributions in the fields of antioxidant treatments and pharmaceuticals. In conclusion, the antioxidant properties of *G. elwesii* should be considered as a source that highlights the importance of natural ingredients for health.

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