İnönü Üniversitesi Sağlık Hizmetleri Meslek Yüksekokulu Dergisi
ISSN: 2147-7892, Cilt 9, Sayı 3 (2021) 781-795
doi: 10.33715/inonusaglik.904506

Journal of Inonu University Health Services Vocational School
ISSN: 2147-7892, Volume 9, Issue 3 (2021) 781-795
doi: 10.33715/inonusaglik.904506

Original Article / Araştırma Makalesi

BIOLOGICAL PROPERTIES AND PHENOLIC CONTENTS OF EXTRACTS PREPARED FROM *ERYNGIUM BILLARDIERI* F. DOLAROCHE

Eryngium billardieri F. Dolaroche'den (Boğa Dikeni) Hazırlanan Ekstraktlarin Fenolik İçerikleri Ve Biyolojik Özellikleri

Ahmet SAVCI[1]
[1]*Muş Alparslan University, Faculty of Science and Art, Muş*

Geliş Tarihi / Received: *28.03.2021* ***Kabul Tarihi / Accepted:*** *13.09.2021*

ABSTRACT

In this study, *Eryngium billardieri* was collected from Patnos district of Ağrı province during the vegetation period. While some of the collected plant was stored fresh in a -86ºC freezer, the other part was preserved as herbarium material. After the species of the plant were identified, methanol and pure water extracts were prepared from the aerial parts of the fresh and herbarium samples. The phenolic contents of the extracts were determined by using HPLC. Moreover, various *in vitro* antioxidant properties and DNA protective properties of the relevant extracts were tested. The comparison of the test results showed that the phenolic compounds of the fresh plant extracts were more than that of herbarium extracts. Although both extracts showed good antioxidant activities, their activity was lower than the standart antioxidants. Moreover, herbarium extracts were seen to have a relatively better effect on the pUC18 DNA stabilization, irrespective of the presence of H_2O_2 in the environment. Having considered that there is limited number of studies on *E.billardieri* in the literature, this study will offer important insights into the studies in this area.

Keywords: Antioxidant, Apiaceae, DNA, *Eryngium billardieri*, HPLC.

ÖZ

Bu çalışmada, *Eryngium billardieri (*Boğa Dikeni) Ağrı iline bağlı Patnos ilçesinden vejetasyon döneminde toplandı. Toplanan bitkinin bir kısmı -86ºC dondurucuda taze olarak depolanırken, diğer bir kısmı ise herbaryum materyali halinde saklandı. Tür tayini yapıldıktan sonra bitkinin taze ve herbaryum numunelerinin toprak üstü kısımlarından metanol ve saf su ekstraktları hazırlandı. Ekstraktların fenolik içeriği HPLC kullanılarak belirlendi. Ayrıca ilgili özütlerin çeşitli *in vitro* antioksidan özellikleri ve DNA koruyucu özellikleri test edildi. Nihayetinde, elde edilen sonuçlar biribiri ile ilişkilendirilmiştir. Araştırma sonuçları taze bitki ekstraktlarının herbaryum ekstraktlarına göre daha zengin fenolik madde içerdiğini gösterdi. Her iki ekstre iyi antioksidan aktivite gösterse de aktiviteleri standart antioksidanlardan daha düşüktü. Ayrıca herbaryum ekstraktlarının ortamdaki H_2O_2 varlığından bağımsız olarak, pUC18 DNA stabilizasyonu üzerinde nispeten daha olumlu bir etkiye sahip olduğu görüldü. Literatürde *E.billardieri* üzerinde yapılan çalışma sayısının sınırlı olduğu göz önüne alınırsa, bu çalışma bu konuda yapılacak çalışmalarda yol gösterici olacaktır.

Anahtar kelimeler: Antioksidan, Apiaceae, DNA, *Eryngium billardieri*, HPLC.

Ahmet SAVCI ✉, *a.savci@alparslan.edu.tr*
Muş Alparslan University, Faculty of Science and Art, Muş

Bu makaleye atıf yapmak için(How to cite this article): Savcı, A. (2021). Biological Properties and Phenolic Contents of Extracts Prepared from *Eryngium Billardieri* F. Dolaroche. İnönü Üniversitesi Sağlık Hizmetleri Meslek Yüksekokulu Dergisi, 9(3), 781-795. doi: 10.33715/inonusaglik.904506

INTRODUCTION

Before the discovery of synthetic drugs, almost all civilizations relied on plants to maintain their health or treat various diseases (Parham et al., 2020; Soumia, 2018). In ancient times, the plants, particularly beneficial for the body, were used in different forms such as expressed fruit juice, powder, boiling tea, flavoring various foods, or infusion (Savci, Kocpinar, Alan, & Kursat, 2020; Soumia, 2018). Although the use of plants has become less attractive after the synthetic drugs, herbal treatment is still widely used by the society in order to strengthen the immune system before or after illness (Soumia, 2018). The healing effects of these plants, which are considered to be pharmacokinetic, are associated with the amount and richness of antioxidants in their structure (Alan et al., 2019; Kikowska, Dworacka, Kędziora, & Thiem, 2016). Due to the presence of phenolics and secondary metabolites, plants have positive effects on neurodegenerative diseases such as Alzheimer's and Parkinson's and common problems such as phlegm, cough, sinusitis, edema, and wound healing (Nebija et al., 2009; Soumia, 2018). Negative psychological factors (e.g., stress, cigarette smoke, UV light, and environmental pollution) are known to cause oxidative stress associated with cancer and neurodegenerative diseases by increasing cellular reactive oxygen species (Budak, Ceylan, Kocpinar, Gonul, & Erdogan, 2014; Budak, Gonul, Ceylan, & Kocpinar, 2014; Kolaç, Gürbüz, & Yetiş, 2017). It is also reported that plant phenolics, many of which are powerful antioxidants, play a protective role against many diseases by establishing the reactive oxygen species (ROS) balance (Bilal, 2020; Savci, et al., 2020; Soumia, 2018).

Apiaceae is one of the largest and cosmopolitan families of angiosperm plants, and this family has been used in the form of medicine, food source, spice and animal feed for many years. 101 of 485 species in the world grow in Turkey, and the endemism rate of this family is 37% (Hançer & Uruşak, 2017). Apiaceae family have rich phenolic content, and the members of this family have been reported to possess positive effects on many diseases and ailments including edema, cough, bronchitis, skin and respiratory ailments, sinusitis, urinary tract infections, wound healing, infertility and inflammatory diseases (Kikowska et al., 2016; Soumia, 2018).

Eryngium genusis is one of the members of the Apiaceae family in Turkey, and it can grow in Eurasia, North Africa, North and South America and Australia (Kikowska et al., 2016). *Eryngium* species are widely used for therapeutic purposes in traditional medicine due to their bioactive components. The aerial parts and roots of this species are used in the treatment of skin

and kidney diseases. It is very useful for digestive problems and headaches. Moreover, the plants of this genus have strong antioxidant, antimicrobial and cytotoxic effects (Ayuso et al., 2020; Benmerache et al., 2016; Rjeibi, Saad, Ncib, & Souid, 2017).

In this study, methanol and pure water extracts were prepared from aerial parts of *Eryngium billardieri,* and their phenolic contents were investigated by HPLC. In addition, *in vitro* antioxidant properties and DNA protective properties of the extracts were tested. The reduction capacities of copper and iron ions were examined by CUPRAC and FRAP methods, respectively. Moreover, DPPH and ABTS radical scavenging activities and lipid peroxidation capacities were investigated. The results obtained from these analyses were correlated with each other.

MATERIAL AND METHOD

Collection of plant and preparation of HPLC samples

Eryngium billardieri were collected from Patnos district of Ağrı province during the vegetation period in 2020. A portion of collected plants was converted into herbarium material with the code A. SAVCİ-1 and the other portion was stored freshly in -86ºC freezer in Muş Alparslan University, Center Research and Application Center. 50 g of both were taken and extracts were prepared by pulverizing with liquid nitrogen. 1 mg/mL fresh extracts were prepared by diluting main extracts for loading to HPLC (Agilent 1260 Infinity II).

Phenolic substance analysis by HPLC

Final concentrations of standards were adjusted to 10 mg/mL. 1% acetic acid and acetonitrile (9:1 ratio respectively) a stock solution was formed by mixing each other and obtained solution were used in the preparation of standards. Methanol was then dissolved in this homogeneous mixture at the rate of 1:1. By diluting the prepared solution, intermediate stock standards were prepared at different concentrations (100 mM, 75 mM, 50 mM, 25 mM and 10 mM) and these were used in the formation of a standard graph (Seal, 2016). Standard chromatograms were formed by HPLC to measuring the concentrations of phenolic in the extracts and standard chromatograms and curves were used to calculate the 17 phenolic concentrations of the extracts. Prepared extracts were diluted 20 mg/mL and filtered using the 0.45 μm membrane filter. HPLC conditions used for testing are shown in Table 1 and phenolic concentrations of the extracts were detected as seen in Table 3.

Table 1. HPLC Operating Conditions And Gradient Elution Program

HPLC operating conditions	
Model	Agilent Technologies 1260 Infinity II
Colon	ACE 5 C18 (250x4.6 mm id)
Kolon Oven	G7130A
Detector	1260 DAD WR
Pump	1260 Quat Pump VL
Mobile phase	A: %1 Acetic Acid
	B: Acetonitrile
Detection	272, 280 ve 310 nm
Autosampler	1260 Vialsampler
Flow Rate	1 mL/dk
Colon Temperature	28 ºC
Injection	20 µl

Gradient elution		
Time (min)	A (%)	B (%)
0	90	10
25	60	40
39	40	60
50	10	90
55	90	10

Preparation of samples for *in vitro* antioxidant studies

Both herbarium material and fresh plant were crushed with the help of liquid nitrogen and incubated in their solvent for 48 hours at room temperature by mixing. The solvents were evaporated under a suitable vacuum with the help of a rotary evaporator (Heidolph). After evaporation, the extracts of the samples were prepared at a concentration of 1 mg / mL and these samples were used in the next levels of the antioxidant assays.

In vitro antioxidant assays

Ferric iron (Fe^{3+}) reduction test by FRAP

Total reduction power was determined according to the slightly modified FRAP method (Oyaizu, 1986). 10, 20 and 30 µl of the stock solutions were taken into test tubes and the volume was completed to 200 µl with distilled water. Then, 500 µl phosphate buffer (pH: 6.6) and 1% [$K_3Fe(CN)_6$] were added to the test tubes. After 20 minutes of incubation at 50ºC, 500 µl trichloracetic acid (TCA) was added to the mixture. 500 µl was taken from the upper phase of the solution and 500 µl of distilled water and 100 µl of $FeCl_3$ were added to them. The absorbances of the samples were measured at 700 nm by using a microplate reader (Thermo scientific MULTISCAN GO).

Cupric Cations (Cu^{2+}) Reduction Activity

The Cu^{+2} reduction capacities of the samples were evaluated using the CUPRAC method which was modified by Gulcin (2006). 250 µl $CuCl_2$ was put into the test tubes. 250 µl neocuprine solution and acetate buffer (pH:6.5) were added to it. Then, different concentrations (10, 20, 30 µl) of compounds and standard samples were added and incubated for 30 minutes.

After the incubation, the absorbance values were measured at 450 nm wavelength using a microplate reader (Thermo scientific MULTISCAN GO).

DPPH free radical scavenging activity

1,1-Diphenyl-2-picryl-hydrazil (DPPH) radical scavenging method was used for the antiradical studies. DPPH free radical scavenging method is one of the most commonly used methods to determine the radical scavenging activities of various factors. The basis of method of Blois (1958) used in this study is based on the reduction of DPPH (1,1-Diphenyl 2-picrylhydrazyl) radical dissolved in the alcohol. After transferring from samples in different concentrations (10, 20, 30 µl) to tubes, the total volume was completed to 600 µl with ethanol. Then 200 µl DPPH radical solution was added to them. After 30 minutes of incubation, the absorbances of the samples were measured at 517 nm using a microplate reader (Thermo scientific MULTISKAN GO). 600 µl ethanol and 200 µl DPPH radical solution were used for control purposes. The radical scavenging activity of the samples was calculated according to the following equation:

$$\text{Scavenging Capacity of DPPH Radical (\%)} = \left(1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of control}}\right) \text{x } 10$$

ABTS radical scavenging activity

Scavenging activity of ABTS (2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) radical was determined according to the method of Wu, Chang, Chen, Fan, and Ho (2009). Firstly, 2.45 mM $K_2S_2O_8$ and 7 mM ABTS solutions were incubated by mixing at equal proportions for 16 hours at room temperature. To obtain desired control absorbance value, ABTS radical solution was diluted by adding ethyl alcohol. Distilled water and the radical solution were transferred into each tube at the same portion (500 µl), and 1 mg/mL of extracts and standards were added at different volumes (10 µL, 20 µL, and 30 µL). After the incubation for 2 hours at room temperature, absorbance values were recorded against the blank phosphate buffer (pH: 7.4). Absorbance values of samples were measured at 734 nm using a microplate reader (Thermo scientific MULTISKAN GO).

DNA protective activity study

DNA protective activity of samples was evaluated by agarose gel electrophoresis (Siddall et al., 2002). 200 mg of the main extracts were dissolved in the 1 mL of DMSO and the stock extract solutions were prepared. Fresh extracts were prepared at 1 mg/mL concentration by diluting the stock extracts in DMSO. Fresh extracts were mixed with the other electrophoresis as shown in Table 2 and incubated for 24 hours at 37 ºC. DNA protective activities of the extracts at 1 mg/mL, 0.5 mg/mL, and 0.25 mg/mL concentrations were evaluated. Agarose gel electrophoresis was operated for 40 volts and 2 hours. The electrophoresis product was stained with ethidium bromide and visualized using BIORAD ChemiDoc XRS Imaging system.

Table 2. The Amounts And Components Of Samples For Electrophoresis

DNA (µL)	H_2O_2 (µL)	DMSO (µL)	Extract (10 µL) and Concentration	PW (µL)	Total Volume (µL)
15	-	-	-	15	30
15	5	-	-	10	30
15	5	10	-	-	30
15	-	10	-	5	30
15	5	-	Sample (0.25 mg/mL)	-	30
15	5	-	Sample (0.5 mg/mL)	-	30
15	5	-	Sample (1 mg/mL)	-	30
15	-	-	Sample (0.25 mg/mL)	5	30
15	-	-	Sample (0.5 mg/mL)	5	30
15	-	-	Sample (1 mg/mL)	5	30

PW: Pure water

RESULTS AND DISCUSSION

Results of phenolic concentration

The phenolic concentration of the extracts was determined from both materials using HPLC, and the results are shown in terms of µg/mL (Table 3). HPLC chromatogram results of the fresh extract are shown in figure 1, and herbarium extract chromatogram results are shown in figure 2. It can be seen from the table that the fresh extract was found to have the highest concentration of transcoumaric acid and the lowest amount of quercetin. Similar to the fresh extract, the herbarium material extract has the highest transcoumaric acid concentration, while the lowest concentration in this case was the rosmarinic acid. It is also obvious that the phenolic concentration of the fresh extract is significantly higher than that of the herbarium material extract. Gallic acid, curcumin, 3,4-Dihydroxybenzoic acid could not be identified in both of the extracts.

E. bilardieri species have not been widely studied in the literature, and there is a lack of information on their phenolic content. Therefore, the results of the current study were compared with the published data on different *Eryngium* species. The previous literature showed that caffeic acid and vanillic acid concentrations were higher in *Eryngium maritimum* (Rjeibi et al., 2017), while 3,4-dihydroxyphenylacetic, cafeic, chlorogenic and rosmarinic acid concentration were much higher in *Eryngium alpinum* L. (Kikowska et al., 2019). The common feature of the three *Eryngium* species was that they have rich phenolic content in all cases. The difference between the results of these studies may be due to the difference in the extraction method and the fresh plant extract having more volatile components.

Table 3. The μg/mL Concentrations Of 17 Different Phenolics In The Eb (*E. Bilardieri*) Extracts

Phenolic name	**Fresh *Eb* Extract**	**Herb. *Eb* Extract**
Ascorbic acid	5.309	-
3,4-Dihydroxybenzoic acid	0.000	0.000
4 hydroxybenzoic acid	2.069	1.208
transcoumaric acid	**17.225**	**8.899**
Myricetin	1.721	1.830
Abscisic acid	2.075	-
Quercetin	**1.584**	-
Apigenin	3.413	-
Kaempferol	1.629	-
Catechol	9.329	1.529
Vanillin	3.705	0.000
Curcumin	-	-
Caffeic acid	1.717	0.000
Cinnamic acid	5.681	3.342
Rosmarinic acid	5.463	**0.866**
Salicylic acid	4.171	0.000
Gallic acid	-	-
Total phenolic concentration	**65.091**	**16.808**

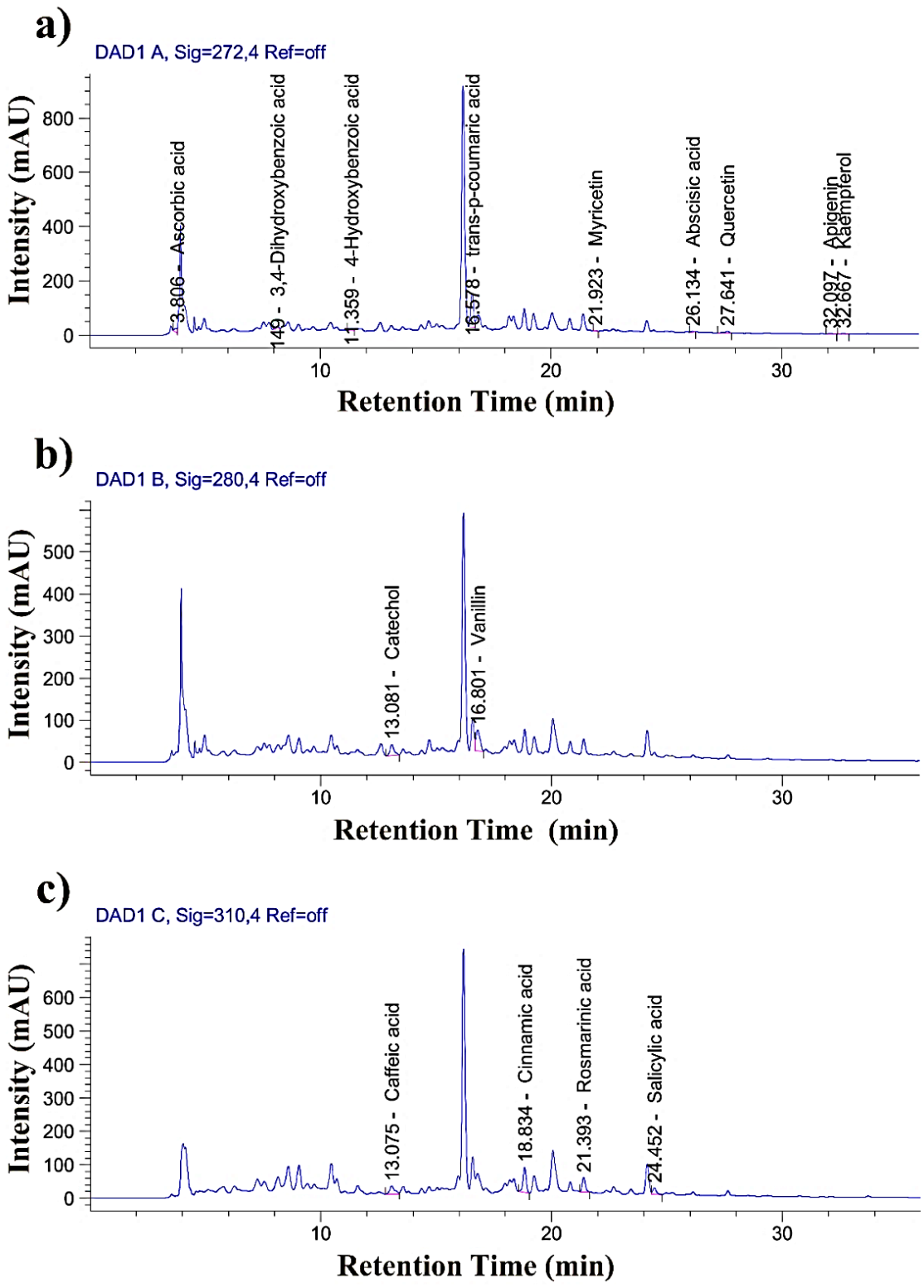

Figure 1. HPLC Phenolic Chromatogram of Fresh *Eryngium Billardieri* Extract

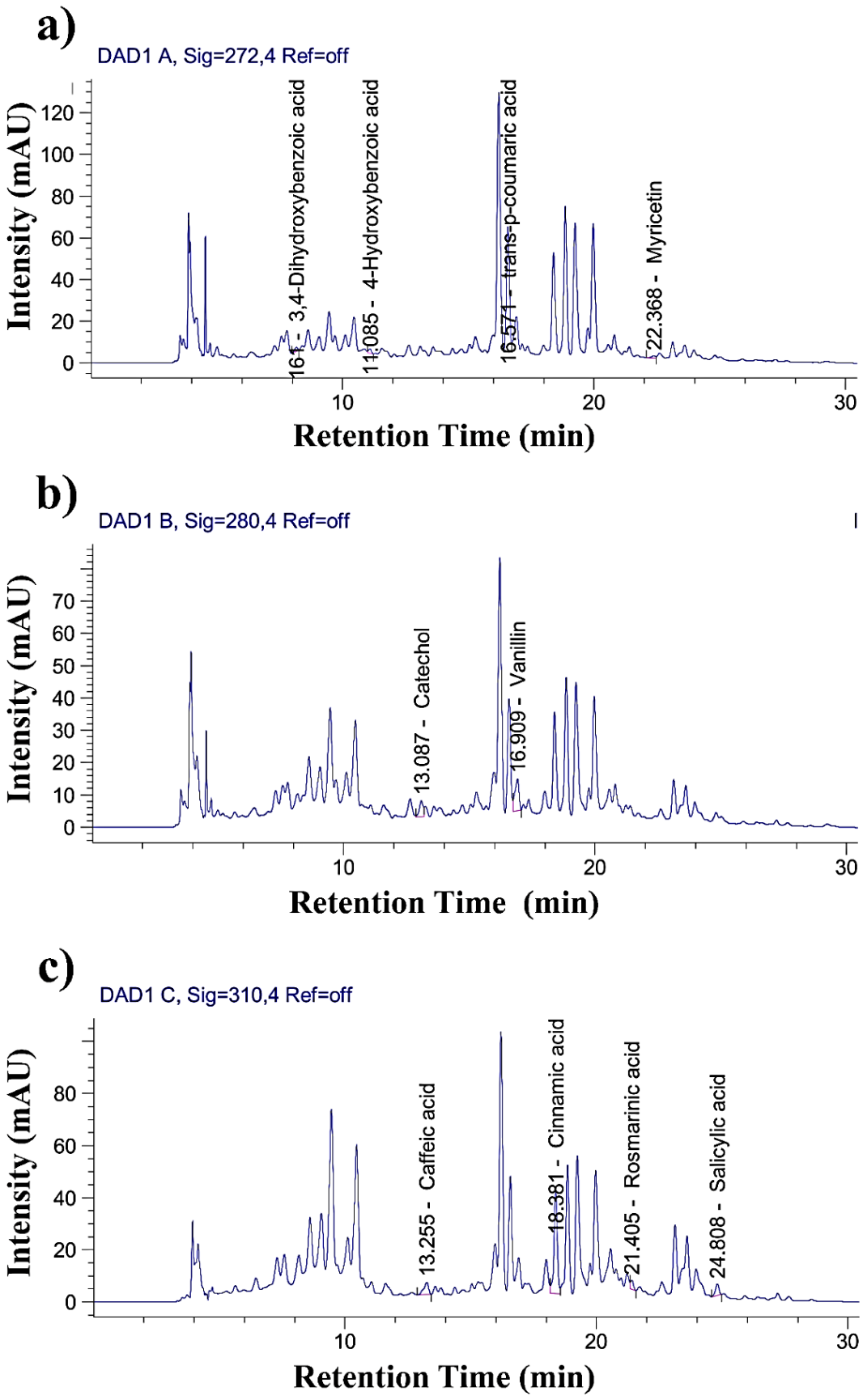

Figure 2. HPLC Phenolic Chromatogram of Herbarium *Eryngium Billardieri* Extracts

Results of *in vitro* antioxidant activity

In the scope of the antioxidant studies, the antioxidant activities of the extracts were compared with the standard antioxidants of butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and ascorbic acid (AA), and the results were expressed as µg TE / ml extract as shown in Table 4. As seen in the results, it was determined that the metal reduction capacities of the extracts obtained from fresh and herbarium material were detected to be low compared to BHA, BHT and AA known as standard antioxidants. The IC_{50} value is the concentration of the sample amount required to convert 50% of the substrate into a product. IC_{50} values of DPPH and ABTS radical reducing activities were observed to be significantly higher than those of standard antioxidants. This indicates that the radical scavenging activities of the extracts are lower than the standards, and the radical scavenging results are consistent with the heavy metal reduction results. Lipid peroxidation scavenging percentages were shown to be significantly lower than the standards, and this is consistent with other antioxidant results. In addition, although the metal reduction capacity of fresh plant methanol extract is higher in CUPRAC results, other results of CUPRAC and FRAP showed that methanol and water extracts prepared from herbarium material had better metal scavenging power than fresh plant extracts. Although a similar effect was observed in the DPPH scavenging capacity of the methanol extract, IC_{50} value of ABTS radical scavenging of methanol extract produced from fresh plant is lower, and this result is proof that fresh herb is a better ABTS scavenger. Contrary to the aforementioned results, lipid peroxidation scavenging percentages of water and methanol extract obtained from the fresh plant were higher than the results of herbarium extracts. This is a sign that the fresh herb is a good lipid peroxidation scavenger. As a result, fresh plant extracts were stronger in the lipid peroxidation scavenging, however, herbarium material was found to be better in other antioxidant results.

Table 4. *In vitro* Antioxidant Activities Of Fresh And Herbarium Material Of *E.Billardieri*. FRAP And CUPRAC Results as µg TE / ml Extract, DPPH And ABTS Results as IC50, And Lipid Peroxidation as %, PW: Pure Water

Samples	FRAP (µg TE/ml extract)	CUPRAC (µg TE/ml extract)	DPPH (IC_{50})	ABTS (IC_{50})	Lipid Peroxidation (%)
Fresh *Eb* (PW Ext.)	7.30±1.53	0.72±0.01	32.72±0.62	134.83±7.42	25.70±0,19
Fresh *Eb* (MetOH Ext.)	18.21±0.89	1.10±0.01	27.08±0.78	20.09±0.25	31.5±0.11
Herbarium *Eb* (PW Ext.)	11.07±2.89	0.97±0.02	28.24±1.34	43.11±2.72	22.6±0.09
Herbarium *Eb* (MetOH Ext.)	30.69±2.13	0.92±0.01	26.78±1.55	44.66±1.83	19.5±0.21
BHA	112.37±0.25	5.47±0.02	14.24±0.11	14.38±0.37	55,2±0.09
BHT	86.24±1.66	4.38±0.02	14.91±0.02	14.35±0.23	52,9±0.02
AA	199.54±1.83	2.29±0.23	12.66±0.06	13.59±0.09	43,6±0.22

Biological studies on *E.billardieri* could not be found in the literature. However, the results of studies with different species belonging to the genus *Eryngium* confirm the results of this study. In a study, it was reported that *E. caucasicum* methanol extracts exhibited very good DPPH radical scavenging and ferric ion reducing activity and the activity increased depending on the concentration increase (Ebrahimzadeh, Nabavi, & Nabavi, 2009). In another study, it was determined that *E. maritimum* water extract had low phenolic content and showed strong ABTS radical scavenging activity (Meot-Duros, Le Floch, & Magné, 2008). According to the results of the study with *Eryngium campestre* L., it has been reported that root ethanol extract is very active in the scavenging of DPPH radicals. In addition, it was observed that ethanol extracts from roots and aerial parts inhibited hydroxyl radicals by 50% and 45%, respectively, and was determined that the aerial parts inhibited the lipid peroxides better than root extract (Nebija et al., 2009). In another study, phenolic and flavonoid contents and *in vitro* antioxidant activities of methanol, acetone, butanol and ethyl acetate extracts from *E. maritimum* root were investigated and acetone extract was reported to have the highest contents of phenolics and flavonoids, and butanol extract was detected to show strong radical scavenging and iron reduction potential (Kholkhal, Ilias, Bekhechi, & Bekkara, 2012). Paun et al. evaluated the biological activities of Eryngium planum extract enriched with polyphenols obtained by nanofiltration. In the FRAP results, the iron reducing power of the extract was found to be higher than AA, while the DPPH radical elimination results were lower than AA (Paun et al., 2019). Since the plant species used in this study is different from the species used in the previous study, it was hoped that there might be some differences in biological activity results however, the results were found to generally support each other.

Results of DNA protective activity

Plasmid DNAs are circular and can contain form I and form II structures. Form I is more stable and moves faster than form II on the agarose gel. However, some molecules such as H_2O_2 can effect negatively and it can destroy form I and form II structures or convert form I into form II and form III (Zhang et al., 2001). In this study, DNA protective activities of the extracts were tested on pUC18 DNA. These effects are shown in figure 3 with the help of lines. The loading components and amounts of the samples are shown in (Table 2). According to this, it is clear that pUC18 has pure form I and form II structures (Line 1). It has been shown that H_2O_2 destroys form I and converts form II into form III (Line 2) and it appears that H_2O_2 and DMSO together or DMSO only destroy pUC18 DNA (Line 3 and Line 4, respectively). Also, the effects of both

fresh and herbarium extracts on pUC18 DNA were investigated in the pure environment (without H_2O_2) and against H_2O_2 and a desired significant effect on pUC18 DNA protection was not observed depending on the concentration of both extracts. It was also observed that both of extracts did not have a significant effect on pUC18 DNA in the pure environment (Line 5, 6, and 7) and could not prevent the damage caused by H_2O_2 (Line 8, 9, and 10). A similar DNA protective effect of the herbarium extract was observed and it can be said that herbarium extract stabilizes relatively pUC18 DNA compared to the those of fresh extract in both environments with H_2O_2 and H_2O_2 free. This result is consistent with the antioxidant results largely, but not with the phenolic concentration results.

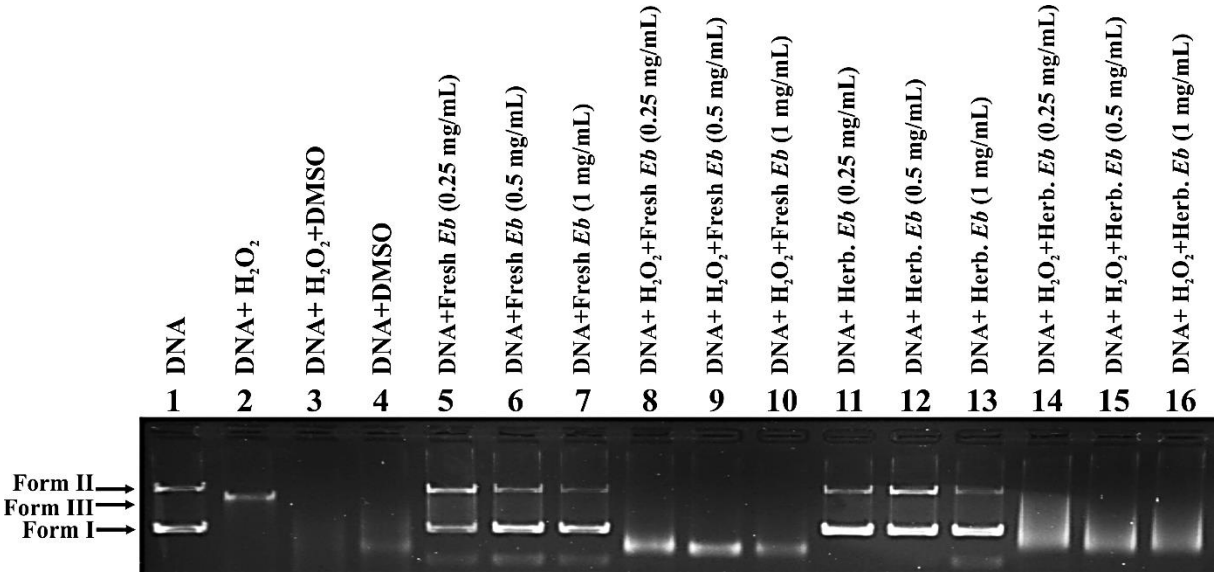

Figure 3. DNA Protective Effects of the Extracts Obtained from the Leaves of Fresh and Herbarium Material *Eryingium Billardieri*, Herb.: Herbarium and *Eb*: *Eryngium Billardieri*, DNA: pUC18, Extract: Pure Water.

There may be various reasons why antioxidant and DNA breakage results do not show parallelism with HPLC results. Only phenolic content was evaluated by HPLC. However, different compounds (flavonoid, organic acid, terpenes, etc.) contained in the substance may cause different results.

CONCLUSION

In this study, methanol and water extracts were prepared from the fresh and herbarium of the *E. billardieri*, and the biological activities of both extracts were studied. The results showed that both extracts showed high antioxidant activity and DNA protective effect. Their phenolic

concentration was different from each other. Herbarium extracts exhibited higher antioxidant activity, while fresh extracts contained higher phenolics. IC50 values of ABTS radical scavenging activities were found to be considerably higher than the standards. Similarly, IC50 values of radical scavenging activities in both extracts were significantly higher than the standards, indicating that the extracts were weak radical scavengers. Unlike the results of heavy metal elimination and radical scavenging, fresh extracts were observed to be a better lipid peroxidation scavenger. In addition, herbarium extracts appeared to have a relatively better effect on the pUC18 DNA stabilization irrespective of the presence of H_2O_2 in the environment. Due to the limited number of studies on *E.billardieri* species in the literature, the results of this study will provide significant insights and fill the gaps in this field.

Acknowledgements

I am grateful to Assoc. Dr. Murat Kürşat from Bitlis Eren University for his contribution in the identification of the plant used in this study.

Conflict Of Interest

I declare no conflict of interest, financial or otherwise.

REFERENCES

Alan, Y., Savcı, A., Koçpınar, E. F., Kurşat, M., Topdemir, S., Karataş, M., Çakmak, B. (2019). Antimicrobial, antioxidant and DNA protective effects and phenolic content of Lallementia canescens (L.) Fisch. & CA Mey. and Lallementia peltata (L.) Fisch. & CA Mey. Biyolojik Çeşitlilik ve Koruma, 12(3), 78-88.

Ayuso, M., Pinela, J., Dias, M. I., Barros, L., Ivanov, M., Calhelha, R. C., ... Gallego, P. P. (2020). Phenolic composition and biological activities of the in vitro cultured endangered Eryngium viviparum J. Gay. Industrial Crops and Products, 148, 112325.

Benmerache, A., Magid, A. A., Berrehal, D., Kabouche, A., Voutquenne-Nazabadioko, L., Messaili, S., ... Kabouche, Z. (2016). Chemical composition, antibacterial, antioxidant and tyrosinase inhibitory activities of glycosides from aerial parts of Eryngium tricuspidatum L. Phytochemistry Letters, 18, 23-28.

Bilal, Ç. (2020). Renal hücrelerde metoreksat kaynaklı sitotoksisite: Kurkumin'in koruyucu rolü. İnönü Üniversitesi Sağlık Hizmetleri Meslek Yüksek Okulu Dergisi, 8(2), 281-292.

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. Nature, 181(4617), 1199.

Budak, H., Ceylan, H., Kocpinar, E. F., Gonul, N., Erdogan, O. (2014). Expression of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in oxidative stress induced by long-term iron toxicity in rat liver. [Research Support, Non-U.S. Gov't]. J Biochem Mol Toxicol, 28(5), 217-223. doi: 10.1002/jbt.21556

Budak, H., Gonul, N., Ceylan, H., Kocpinar, E. F. (2014). Impact of long term Fe(3)(+) toxicity on expression of glutathione system in rat liver. [Research Support, Non-U.S. Gov't]. Environ Toxicol Pharmacol, 37(1), 365-370. doi: 10.1016/j.etap.2013.12.007

Ebrahimzadeh, M. A., Nabavi, S. F., Nabavi, S. M. (2009). Antioxidant activity of leaves and inflorescence of Eryngium caucasicum Trautv at flowering stage. Pharmacognosy Research, 1(6), 435.

Gulcin, I. (2006). Antioxidant activity of caffeic acid (3,4-dihydroxycinnamic acid). [Comparative Study]. Toxicology, 217(2-3), 213-220. doi: 10.1016/j.tox.2005.09.011

Hançer, Ç. K., Uruşak, E. A. (2017). Apiaceae familyası meyve anatomisindeki "Vitta" terimi ve yerleşimleri. Avrasya Terim Dergisi, 5(2), 19-24.

Kholkhal, W., Ilias, F., Bekhechi, C., Bekkara, F. A. (2012). Eryngium maritimum: A rich medicinal plant of polyphenols and flavonoids compounds with antioxidant, antibacterial and antifungal activities. Current Research Journal of Biological Sciences, 4(4), 437-443.

Kikowska, M., Dworacka, M., Kędziora, I., Thiem, B. (2016). Eryngium creticum-ethnopharmacology, phytochemistry and pharmacological activity. A review. Revista Brasileira de Farmacognosia, 26(3), 392-399.

Kikowska, M., Thiem, B., Szopa, A., Klimek-Szczykutowicz, M., Rewers, M., Sliwinska, E., Ekiert, H. (2019). Comparative analysis of phenolic acids and flavonoids in shoot cultures of Eryngium alpinum L.: an endangered and protected species with medicinal value. Plant Cell, Tissue and Organ Culture (PCTOC), 139(1), 167-175.

Kolaç, T., Gürbüz, P., Yetiş, G. (2017). Doğal ürünlerin fenolik içeriği ve antioksidan özellikleri. İnönü Üniversitesi Sağlık Hizmetleri Meslek Yüksek Okulu Dergisi, 5(1), 26-42.

Meot-Duros, L., Le Floch, G., Magné, C. (2008). Radical scavenging, antioxidant and antimicrobial activities of halophytic species. Journal of Ethnopharmacology, 116(2), 258-262.

Nebija, F., Stefkov, G., Karapandzova, M., Stafilov, T., Panovska, T. K., Kulevanova, S. (2009). Chemical characterization and antioxidant activity of Eryngium campestre L., Apiaceae from Kosovo. Македонско фармацевтско друштво, ул. Маршал Тито 13б/8, Скопје, Македонија Macedonian Pharmaceutical Society, Marshal Tito 13b/8, Skopje Macedonia, 23.

Oyaizu, M. (1986). Studies on products of browning reaction: antioxidative activity of products of browning reaction. Jpn. J. Nutr, 44(6), 307-315.

Parham, S., Kharazi, A. Z., Bakhsheshi-Rad, H. R., Nur, H., Ismail, A. F., Sharif, S., ... Berto, F. (2020). Antioxidant, Antimicrobial and Antiviral Properties of Herbal Materials. Antioxidants, 9(12), 1309.

Paun, G., Neagu, E., Moroeanu, V., Albu, C., Savin, S., Lucian Radu, G. (2019). Chemical and bioactivity evaluation of Eryngium planum and Cnicus benedictus polyphenolic-rich extracts. BioMed Research International, 2019.

Rjeibi, I., Saad, A. B., Ncib, S., Souid, S. (2017). Phenolic composition and antioxidant properties of Eryngium maritimum (sea holly). J Coast Life Med, 5(5), 212-215.

Savci, A., Kocpinar, E. F., Alan, Y., Kursat, M. (2020). Antioxidant, antimicrobial, and DNA protection activities of some Tanacetum species and phenolic richness in their ethanolic extracts. International Food Research Journal, 27(1), 160-170.

Seal, T. (2016). Quantitative HPLC analysis of phenolic acids, flavonoids and ascorbic acid in four different solvent extracts of two wild edible leaves, Sonchus arvensis and Oenanthe linearis of North-Eastern region in India. Journal of Applied Pharmaceutical Science, 6(2), 157-166.

Siddall, T. L., Ouse, D. G., Benko, Z. L., Garvin, G. M., Jackson, J. L., McQuiston, J. M., ... VanHeertum, J. C. (2002). Synthesis and herbicidal activity of phenyl-substituted benzoylpyrazoles. Pest Management Science: Formerly Pesticide Science, 58(12), 1175-1186.

Soumia, B. (2018). Eryngium campestre L.: Polyphenolic and flavonoid compounds; Applications to health and disease. Polyphenols: Mechanisms of Action in Human Health and Disease, 69-79.

Wu, L.-c., Chang, L.-H., Chen, S.-H., Fan, N.-c., Ho, J.-a. A. (2009). Antioxidant activity and melanogenesis inhibitory effect of the acetonic extract of Osmanthus fragrans: A potential natural and functional food flavor additive. LWT-Food Science and Technology, 42(9), 1513-1519.

Zhang, H., Barcelo, J. M., Lee, B., Kohlhagen, G., Zimonjic, D. B., Popescu, N. C., Pommier, Y. (2001). Human mitochondrial topoisomerase I. Proc Natl Acad Sci U S A, 98(19), 10608-10613. doi: 10.1073/pnas.191321998