KSÜ Tarım ve Doğa Derg 26 (2), 227-233, 2023
KSU J. Agric Nat 26 (2), 227-233, 2023
https://doi.org/10.18016/ksutarimdoga.vi.1015574

Evaluation of Free Radical Scavenging, Anti-inflammatory and Hypoglycemic Activity of *Helianthemum oelandicum* subsp. *incanum* (Willk.) G. Lopez from Türkiye

Aslı CAN AGCA[1], Sezen YILMAZ SARIALTIN[2], Betül SEVER YILMAZ[3]

[1]Ankara Yıldırım Beyazıt University, Institute of Public Health, Department of Traditional, Complementary and Integrative Medicine, Ankara, TÜRKİYE. [2]Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Ankara, TÜRKİYE. [3]Ankara University, Faculty of Pharmacy, Department of Pharmacognosy, Ankara, TÜRKİYE.

[1] https://orcid.org/0000-0002-5710-3479, [2] https://orcid.org/0000-0002-8387-4146, [3] https://orcid.org/0000-0003-2084-9514

✉: aslicanagca@aybu.edu.tr

ABSTRACT

Helianthemum is represented by 19 taxa, and out of four are endemic in Türkiye. The antioxidant, anti-inflammatory, and hypoglycemic activities of the ethanol (75%) and aqueous extracts from aerial parts of *Helianthemum oelandicum* subsp. *incanum* growing in Türkiye was examined in this study. The water extract exhibited antioxidant activity with 67.26±0.49 and 68.08±0.41 µg ml-1 values on DPPH and ABTS radicals, respectively. The antioxidant activity value of ethanol extract on DPPH radical was found as 46.71±0.27 µg ml-1, where as this value was determined as 51.60±0.76 µg ml-1 for scavenging effects on ABTS radical. Both ethanol and aqueous extracts possessed in vitro anti-inflammatory activity by inhibiting erythrocyte membrane hemolysis in a concentration dependent manner and ethanol extracts exhibited greater activity than aqueous extracts. By measuring the inhibitory effect of the extracts on α-glucosidase, in vitro hypoglycemic activity was assessed. According to the results of this study, the IC_{50} values of both ethanolic and aqueous extracts were found to be close to each other, 2.52±0.01 and 3.21±0.01 µg ml-1, respectively and compared with the standard compound acarbose (IC_{50}= 0.90±0.01 µg ml-1) it was determined that both extracts exhibited strong inhibition on α-glucosidase. Based on all results evaluated, the ethanolic extract displayed higher antioxidant, anti-inflammatory, and hypoglycemic activities than the aqueous extract.

Biochemistry

Research Article

Article History
Received : 27.10.2021
Accepted : 03.03.2022

Keywords
Free radical scavenging activity
Anti-inflammatory activity
Hypoglycemic activity
Helianthemum oelandicum subsp. *incanum*

Türkiye'de Yetişen *Helianthemum oelandicum* subsp. *incanum* (Willk.) G. Lopez Bitkisinin Serbest Radikal Süpürücü, Anti-inflamatuvar ve Hipoglisemik Aktivitelerinin Değerlendirilmesi

ÖZET

Helianthemum Türkiye'de 4'ü endemik olmak üzere 19 takson ile temsil edilmektedir. Bu çalışmada Türkiye'de doğal olarak yetişen *Helianthemum oelandicum* subsp. *incanum* toprak üstü kısımlarının etanollü (%75) ve sulu ekstrelerinin antioksidan, anti-inflamatuvar ve hipoglisemik aktiviteleri araştırılmıştır. Sulu ekstrenin DPPH ve ABTS radikalleri üzerine gösterdiği süpürücü etki miktarı sırasıyla 67.26±0.49 ve 68.08±0.41 µg ml-1 olarak tespit edilmiştir. Bitkinin etanollü ekstresinin DPPH radikaline karşı gösterdiği antioksidan aktivite değeri 46.71±0.27 µg ml-1 iken, ABTS radikali için bu değer 51.60±0.76 µg ml-1 olarak bulunmuştur. Bitkinin hem etanollü hem de sulu ekstresinin eritrosit membran hemolizini konsantrasyona bağlı olarak inhibe ederek in vitro anti-inflamatuvar etki gösterdiği ve etanollü ekstrenin sulu ekstreye göre daha güçlü etkiye sahip olduğu belirlenmiştir. Bitki ekstrelerinin in vitro hipoglisemik aktivite tayini için α-glukozidaza karşı inhibisyon etkileri ölçülmüş, etanollü ve sulu ekstrelerin IC_{50} değerleri sırasıyla 2.52±0.01 ve 3.21±0.01 µg ml-1 bulunmuş ve standart bileşik akarboz ile karşılaştırıldığında (IC_{50}= 0.90±0.01 µg ml-1) α-glukozidaza karşı güçlü inhibisyon gösterdikleri tespit edilmiştir. Tüm aktivite sonuçları bir arada değerlendirildiğinde etanollü ekstrenin antioksidan, anti-inflamatuvar ve hipoglisemik

Biyokimya

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 27.10.2021
Kabul Tarihi : 03.03.2022

Anahtar Kelimeler
Serbest radikal süpürücü aktivite
Anti-inflamatuvar aktivite
Hipoglisemik aktivite
Helianthemum oelandicum subsp. *incanum.*

aktivitesinin sulu ekstreden daha yüksek olduğu bulunmuştur.

Atıf Şekli: Can Agca, A., Yılmaz Sarıaltın, S. & Sever Yılmaz, B. (2023). Türkiye'de Yetişen Helianthemum oelandicum subsp. incanum (Willk.) G. Lopez Bitkisinin Serbest Radikal Süpürücü, Anti-inflamatuvar ve Hipoglisemik Aktivitelerinin Değerlendirilmesi *KSÜ Tarım ve Doğa Derg 26*(2), 227-233. https://doi.org/10.18016/ksutarimdoga. vi.1015574.

To Cite: Can Agca, A., Yılmaz Sarıaltın, S. & Sever Yılmaz, B. (2023). Evaluation of Free Radical Scavenging, Anti-inflammatory and Hypoglycemic Activity of Helianthemum oelandicum subsp. incanum (Willk.) G. Lopez from Türkiye. *KSU J. Agric Nat 26* (2), 227-233. https://doi.org/10.18016/ksutarimdoga.vi.1015574.

INTRODUCTION

Herbal drugs have been popular in the healthcare system globally day by day. With the increasing use of medicinal plants, the pharmaceutical industry focuses on new natural sources. Of course, traditional therapy, which is closely linked to cultures, should be supported by scientific studies so that it can be an option as a complementary medicine and could be suggested by healthcare professionals.

Helianthemum taxa, belonging to the Cistaceae family, are annual or perennial shrub and herbaceous plants that spread widely in Europe, America, North Africa and Central Asia. *Helianthemum* is represented by 19 taxa, and out of four are endemic in Türkiye (Davis, 1965; Şam Gökşen & Baydemir, 2016).

The traditional uses of one of *Helianthemum* species, called *H. nummularium* (L.) Miller., are recorded against constipation and as a blood stopper in Türkiye (Baytop,1999). Besides, *Helianthemum* species are used traditionally as anti-inflammatory, antiulcerogenic, wound healing, antimicrobial, cytotoxic and antidiabetic agent in different countries. The decoctions and teas prepared from this genus are also used to treat gastrointestinal problems (Rubio-Moraga et al., 2013; Şam Gökşen & Baldemir, 2016; Hamza et al., 2019). Djemam et al. determined that *H.lippi* performed relaxation on distal rat colon (Djemam et al., 2020).

There were reports on the antioxidant, antimicrobial, antiprotozoal, antigiardial activities of some *Helianthemum* species (Barbosa et al., 2006; Rubio-Moraga et al., 2013; Baldemir et al., 2017; Benabdelaziz et al., 2017; Chemam et al., 2017) but there was only one study investigating the antidiabetic activity of one of *Helianthemum* genus, called *H. getulum* Pomel an endemic plant to North Sahara (Terfassi et al., 2021). The antidiarrhoeal activity was reported for flavonoids from *H.glomeratum*, an endemic plant grown in Mexico (Calzada & Alanis, 2007).

As the number of studies on biological activities of *Helianthemum* taxa are limited and records on traditional use of *H. oelandicum* subsp. *incanum* (Willk.) G. Lopez (the synonym name: *H.canum* (L.) Miller.) is not found during the literature survey; the goal of this study was to examine the antioxidant, anti-inflammatory and hypoglycemic properties of different extracts from aerial parts of *Helianthemum oelandicum* subsp. *incanum* growing in Türkiye. All assays were carried out on both aqueous and ethanolic extracts in order to determine which could potentially be a natural source for the treatment of diabetes and inflammation.

MATERIAL and METHODS

Plant Material

The aerial parts of *H. oelandicum* subsp. *incanum* growing in Türkiye was collected from Ayaş in Ankara. The plant was identified by Prof. Dr. H. Duman from the Department of Biology, Faculty of Art and Science, Gazi University, Ankara, Türkiye. The voucher specimens are deposited at the Herbarium belonging to the Faculty of Pharmacy of Ankara University with the corresponding herbarium number; AEF27028.

Preparation of Extracts

From the air-dried and powdered aerial parts of *Helianthemum oelandicum* subsp. *incanum*, two different extracts were prepared by using ethanol (75%) and water separately.

The air-dried and powdered aerial parts of the *H. oelandicum* subsp. *incanum* used in this study was weighed accurately and then extracted with ethanol (75%). It was prepared by maceration of 50 g of plant powder in 300 ml of ethanol for 8 hours in 3 days. Each maceration was developed with fresh solvent (3x300 ml). The macerates obtained with ethanol were filtered and evaporated until dry.

Additionally, the air-dried and powdered aerial part of the plant used in this study was weighed accurately and then extracted with water. It was prepared by maceration of 50 g of plant powder in 300 ml of water for 8 hours in 3 days. Each maceration was developed with fresh solvent (3x300 ml). The macerates obtained with water were filtered and lyophilized.

Antioxidant Activity

DPPH Free Radical Scavenging Activity

To determine the effectiveness of plant extracts in scavenging DPPH, they were tested for their ability to neutralize the radical (Okur et al., 2018; Blois et al., 1958). 100 µM DPPH was mixed in methanol and an

array of crude extract concentration (final concentrations: 10-100µg ml^{-1}). Incubation at room temperature in the dark for 30 minutes was performed. Then, the absorbance was measured at 517 nm. Based on the radical reduction percentage, scavenging activity was calculated. Butylated hydroxytoluene (BHT) was served as a standard compound, where as the only solvent was the negative control. Each experiment was performed at least in duplicate. The formula below was used to calculate the percentage of inhibition.

$Inhibition\ (\%) = [(Optical\ density_{control} - Optical\ density_{test\ sample}) / Optical\ density_{control})]x100$ (1).

For each extract, the IC_{50} values were derived from calibration curves nd the results were presented as mean IC_{50}.

ABTS Free Radical Scavenging Activity

The antioxidant activity of the samples was examined by $ABTS^{\cdot+}$ radical cation decolorization assay (Yalcin et al., 2020; Re et al., 1999). 7mM $ABTS^{+}$ aqueous solution was reacted with 2.45 mM potassium persulfate in order to obtain $ABTS^{\cdot+}$. Prior to use, the mixture was kept overnight (12 to 16 hours) at room temperature (in the dark). Fresh $ABTS^{\cdot+}$ solution was prepared for each analysis to prevent self-degradation of the radical. The working solution was diluted with ethanol (pH=7.4) to give an absorbance of 0.700 at 734 nm, and the test compound was dissolved with the final working solution (100x). The final concentration was 1-100µg ml^{-1}. Incubation at room temperature for 6 minutes in the dark was performed. Then, the absorbance was measured at 734 nm. Trolox was used as a standard compound where, as only solvent was the negative control. Each experiment was performed at least in duplicate. The formula below was used to calculate the percentage of inhibition.

$Inhibition\ (\%) = [(Optical\ density_{control} - Optical\ density_{test\ sample}) / Optical\ density_{control})]x100$ (2).

For each extract, the IC_{50} values were derived from calibration curves and the results were presented as mean IC_{50}.

Anti-inflammatory Activity

The anti-inflammatory activity of the extracts was assessed by measuring their protective effects on the human erythrocyte membrane (Shinde et al., 1999; Gunathilake et al., 2018). *The study protocol was confirmed by Human Research Ethics Committees of the Faculty of Medicine of Ankara University (Document date: 14.05.2020, document number: i5-273-20).* Human blood samples were collected from a volunteer who had not taken anti-inflammatory or steroidal medicine for two weeks prior to the experiment. The tubes containing blood samples were centrifuged for 10 min at 3000 rpm. Then the isolated packed cells were washed with 0.85% isosaline (pH 7.2). The cell suspension was prepared using sterile saline solution (1:10). The reaction mixture consisted of an equal volume of test sample (ethanolic and aqueous extracts) and 10% cell suspension. Incubation at 56°C for 30 minutes was performed. After the tubes cooled to room temperature, centrifuge step was performed at 2500 rpm for 5 min. The absorbance of the supernatant was measured at 560 nm. Acetylsalicylic acid (ASA) served as a standard compound, whereas only solvent was used as a negative control. The formula below was used to calculate the percentage of protection.

$Protection\ (\%) = 100-[(Optical\ density_{test\ sample} / Optical\ density_{control}) \times 100]$ (3).

For each extract, the IC_{50} values were derived from calibration curves and the results were presented as mean IC_{50} as well as percentage of protection.

Hypoglycemic Activity

α-glucosidase inhibitory activity was evaluated to determine hypoglycemic activity of the extracts using the method with slight modifications, in vitro (Liu et al., 2014). α-glucosidase (0.02 unit $µl^{-1}$) was mixed with sample (final concentration: 0.10-10µg ml^{-1}) and sodium phosphate buffer (pH 6.8).The reaction mixture was mixed and incubated at 37°C for 20 min. p-nitrophenyl-α-D-glucopyranoside (pNPG) substrate solution was prepared in 0.2 M sodium phosphate buffer (pH=6.8). Then, pNPG substrate solution was added (0.02 M). Incubation at 37°C for 30 minutes was performed. A solution of 0.2 M Na_2CO_3 was added to stop the reaction. Acarbose served as a standard compound. The absorbance was measured at 405 nm. The formula below was used to calculate the percentage of inhibition.

$Inhibition\ (\%) = [(Optical\ density_{control} - Optical\ density_{test\ sample}) / Optical\ density_{control})]x100$ (4).

For each extract, the IC_{50} values were derived from calibration curves and the results were presented as mean IC_{50}.

Statistical Analysis

Statistical analysis was conducted using SPSS 23.0 software. The results were presented as mean IC_{50}± Standard Deviation (SD). One-way analysis of variance was assessed by ANOVA with LSD test to compare the results. A *p*-value below *0.05* was confirmed as the minimum level of significance.

RESULT and DISCUSSION

Antioxidant Activity

DPPH Free Radical Scavenging Activity

Both ethanol and aqueous extracts displayed moderate antioxidant effect by scavenging DPPH free

radical in a concentration-dependent manner. Ethanol extracts showed higher DPPH scavenging activity than aqueous extracts as presented in Table 1.

ABTS Free Radical Scavenging Activity

Ethanol and aqueous extracts noted moderate antioxidant effect by scavenging ABTS free radical. Inhibition increased with the extract concentration. Ethanol extracts displayed greater ABTS free radical scavenging activity than aqueous extracts, as exhibited in Table 2.

In the present study, the water extract exhibited antioxidant activity with 67.26 and 68.08 µg ml^{-1} IC_{50} values on DPPH and ABTS radicals, respectively. The antioxidant activity value of ethanol extract on DPPH radical was found as 46.71 µg ml^{-1}, whereas this value was determined as 51.60 µg ml^{-1} for scavenging effects on ABTS radical.

Table 1 DPPH free radical scavenging activity of the extracts
Çizelge 1 Ekstrelerin DPPH serbest radikal süpürücü etkisi

	Inhibition Concentration$_{50}$ (IC_{50}) (µg ml^{-1}) *İnhibisyon Konsantrasyonu$_{50}$*
Ethanolic extract of H. oelandicum subsp. incanum ***H. oelandicum subsp. incanum etanollü ekstresi***	46.71±0.27[a]
Aqueous extract of H. oelandicum subsp. incanum ***H. oelandicum subsp. incanum sulu ekstresi***	67.26±0.49[a]
Butylated Hydroxytoluene (BHT) ***Butilhidroksi toluen***	11.02±0.44

([a]) Statistically significant: p<0.05, compared to control. The data shows mean±SD of 2-4 independent experiments
([a]) İstatistiksel olarak anlamlı: p<0.05, kontrole kıyasla. Veriler 2-4 bağımsız deneyin ortalama±SS'sini gösterir.

Table 2 ABTS free radical scavenging activity of the extracts
Çizelge 2 Ekstrelerin ABTS serbest radikal süpürücü etkisi

	Inhibition Concentration$_{50}$ (IC_{50}) (µg ml^{-1}) *İnhibisyon Konsantrasyonu$_{50}$*
Ethanolic extract of H. oelandicum subsp. incanum ***H. oelandicum subsp. incanum etanollü ekstresi***	51.60±0.76[a]
Aqueous extract of H. oelandicum subsp. incanum ***H. oelandicum subsp. incanum sulu ekstresi***	68.08±0.41[a]
Trolox (Troloks)	1.58±0.01

([a]) Statistically significant: p<0.05, compared to control. The data shows mean±SD of 2-4 independent experiments.
([a]) İstatistiksel olarak anlamlı: p<0.05, kontrole kıyasla. Veriler 2-4 bağımsız deneyin ortalama±SS'sini gösterir.

Baldemir et al. observed that methanol and water extracts of *H. oelandicum* subsp. *incanum* were rich in phenolic compounds and had scavenging effects on both DPPH and ABTS radicals. Besides, they mentioned the high correlation between scavenging effects and the total phenol content of the extracts (Baldemir et al., 2017). The leaves of the most common 11 *Helianthemum* taxa in Spain were studied for determining the chemical composition and biological activities and reported as *Helianthemum cinereum*, *H. alypoides* and *H. marifolium* constantly showed the greatest radical scavenging activity, additionally the EC_{50} value of radical scavenging activity of water extracts of *H. oleandicum* subsp. *incanum* on DPPH and ABTS radicals was reported as 43.72 µg ml^{-1} and 96.15 µg ml^{-1}, respectively. In 80% MeOH extracts, EC_{50}, values against DPPH and ABTS are found in order of 66.2 µg ml^{-1} and 34 µg ml^{-1} (Rubio-Moraga et al., 2013). Phenolic compounds are known as natural antioxidants, and there were studies showing that the amounts of them were depended on extraction parameters (Sultana et al., 2009; Anokwuru et al., 2011; Demir et al, 2019; Demir et al., 2020), and also a strong correlation between phenolic content of extracts, the molecular structure of compounds and antioxidant activity was drawn attention in previous studies (Rubio-Moraga et al., 2013; Benabdelaziz et al., 2017; Baldemir et al., 2017; Chemam et al., 2017).

Anti-inflammatory Activity

Both ethanol and aqueous extracts possessed *in vitro* anti-inflammatory activity by inhibiting heat-induced erythrocyte membrane hemolysis. The protective effects of the extracts were enhanced as their concentration increased, as shown in Figure 1.

Ethanol extracts showed the highest percentage of inhibition at 20 mg ml^{-1} with 91.33% followed by 10 mg ml^{-1} with 90.97%. Aqueous extracts showed 42.74% and 70.48% inhibition at 10 and 20 mg ml^{-1}, respectively. Ethanol extracts exhibited most significant activity than aqueous extracts, as seen in Table 3.

Water extract of *H. oelandicum* subsp. *incanum* was noted lower anti-inflammatory activity than ethanolic extract (IC_{50}=11.49 and 6.07 mg ml^{-1}, respectively).

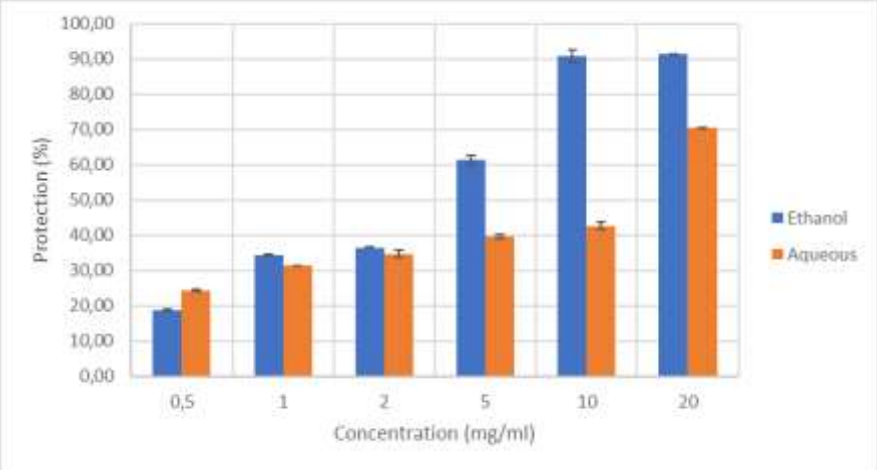

Figure 1 The protective effects of the extracts in different concentrations
Şekil 1 Ekstrelerin farklı konsantrasyonlardaki koruyucu etkileri

Table 3 Anti-inflammatory activity of the extracts
Çizelge 3 Ekstrelerin anti-inflamatuvar aktivitesi

	Inhibition Concentration$_{50}$ (IC_{50}) (mg ml^{-1}) *İnhibisyon Konsantrasyonu$_{50}$*
Ethanolic extract of H. oelandicum subsp. incanum *H. oelandicum subsp. incanum etanollü ekstresi*	6.07±0.12[a]
Aqueous extract of H. oelandicum subsp. incanum *H. oelandicum subsp. incanum sulu ekstresi*	11.49±0.01[a]
Acetylsalicylic acid (ASA) *Asetilsalisilik asit*	0.28±0.01

[a] Statistically significant: $p<0.05$, compared to control. The data shows mean±SD of 2-4 independent experiments.
[a] İstatistiksel olarak anlamlı: $p<0.05$, kontrole kıyasla. Veriler 2-4 bağımsız deneyin ortalama±SS'sini gösterir.

Hypoglycemic Activity

The extracts were tested for hypoglycemic activity using in vitro assessments of α-glucosidase inhibitory activity. Ethanol extracts exhibited higher α-glucosidase inhibitory potential than aqueous extracts. The IC_{50} values of both ethanolic and aqueous extracts were found to be close to each other, 2.52 and 3.21 µg ml^{-1}, respectively and compared with the standard compound acarbose (IC_{50}=0.90 µg ml^{-1}). It was determined that both extracts exhibited strong inhibition on α-glucosidase as shown, in Table 4.

Terfassi et al. studied a different *Helianthemum* taxa called *H. getulum* an endemic plant to septentrional Sahara. They mentioned that both the fractions and extract of plant showed a remarkable inhibition activity on α-glucosidase and were in agreement with the present study (Terfassi et al, 2021).

CONCLUSION

In this study, we tested two extracts prepared with water and ethanol from *Helianthemum oelandicum* subsp. *incanum* from Türkiye for investigating the *in vitro* antioxidant, anti-inflammatory and hypoglycemic activities in the light of traditional uses of the plant worldwide. Present results indicate that although aqueous and ethanolic extracts of the aerial part of the plant were found to show moderate antioxidant and anti-inflammatory effects *in vitro*, both extracts of aerial parts of *H. oelandicum* subsp. *incanum* have potential α-glucosidase inhibitory activities supporting the traditional use against diabetes. Further studies are needed to determine the mechanisms of action and chemical profile of the plant to decide if the plant could be a promising candidate for the treatment of diabetes.

Author's Contribution

The authors declare that they have contributed equally to the article.

Statement of Conflict of Interest

Authors have no conflict of interest to declare.

Statement of Research and Publication Ethics

The authors declare that this study has been performed in accordance with research and publication ethics.

Table 4 α-glucosidase inhibitory activity of the extracts
Çizelge 4 Ekstrelerin α-glukozidaz inhibisyon aktivitesi

	Inhibition Concentration$_{50}$ (IC_{50}) ($\mu g\ ml^{-1}$) ***İnhibisyon Konsantrasyonu$_{50}$***
Ethanolic extract of H. oelandicum subsp. incanum ***H. oelandicum subsp. incanum etanollü ekstresi***	2.52±0.01[a]
Aqueous extract of H. oelandicum subsp. incanum ***H. oelandicum subsp. incanum sulu ekstresi***	3.21±0.01[a]
Acarbose ***Akarboz***	0.90±0.01

([a]) Statistically significant: p<0.05, compared to control. The data shows mean±SD of 2-4 independent experiments.
([a]) İstatistiksel olarak anlamlı: p<0.05, kontrole kıyasla. Veriler 2-4 bağımsız deneyin ortalama±SS'sini gösterir.

ACKNOWLEDGMENTS

The authors would like to thank to Prof. Dr. Hayri Duman for identification of the plant.

REFERENCES

Anokwuru, C.P., Anyasor, G.N., Ajibaye, O., Fakoya, O. & Okebugwu, P. (2011). Effect of extraction solvents on phenolic, flavonoid and antioxidant activities of three nigerian medicinal plants. *Natural and Science, 9*(7), 53-61.

Baldemir, A., Gokşen, N., Ildız, N., Şeker Karatoprak, G. & Koşar, M. (2017). Phytochemical profile and biological activities of *Helianthemum canum* L. BAUMG. from Turkey. *Chemistry & Biodiversity*, *14*, 2-12.

Blois, M.S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature, 26*, 1199-1200. https://doi.org/10.1002/cbdv.201700052

Barbosa, E., Calzada, F. & Campos, R (2006). Antigiardial Activity of Methanolic Extracts from *Helianthemum glomeratum* Lag. and *Rubus coriifolius* Focke in Suckling Mice CD-1. *Journal of Ethnopharmacology 108*, 395–397. https://doi.org/ 10.1016/j.jep.2006.05.026

Baytop, T. (1999). *Türkiye'de Bitkilerle Tedavi (Therapy with Medicinal Plants in Turkey),* Nobel Tıp Basımevi, İstanbul.

Benabdelaziz, I., Marcourt, L., Benkhaled, M., Wolfender, J.L. & Haba, H (2017). Antioxidant and antibacterial activities and polyphenolic constituents of *Helianthemum sessiliflorum* Pers. *Natural Product Research, 31*(6), 686-690. https://doi.org/10.1080/14786419.2016.1209669

Calzada, F. & Alanis, A.D. (2007). Additional antiprotozoal flavonol glycosides of the aerial parts of *Helianthemum glomeratum*. *Phytotherapy Research, 21*, 78-80. doi: 10.1002/ptr.2031

Chemam, Y., Benayache, S., Marchioni, E., Zhao, M., Mosset, P. & Benayache, F (2017). On-Line screening, isolation and identification of antioxidant compounds of *Helianthemum ruficomum*. *Molecules, 22*(239), 1-14. https://doi. org/10.3390/molecules22020239

Davis, P.H. (1965). *Flora of Turkey and East Aegean Islands,* Vol I, Edinburgh University Press, Edinburgh, UK.

Demir, T., Akpınar, Ö., Kara, H. & Güngör, H. (2019). Nar (*Punica granatum* L.) kabuğunun in vitro antidiyabetik, antienflamatuar, sitotoksik, antioksidan ve antimikrobiyal aktivitesi. *Akademik Gıda, 17*(1), 61-71. https://doi.org/ 10.24323/akademik-gida.544647

Demir, T., Akpınar, Ö., Kara, H. & Güngör, H. (2020). Cherry stem phenolic compounds: Optimization of extraction conditions and in vitro evaluations of antioxidant, antimicrobial, antidiabetic, anti-inflammatory, and cytotoxic activities. *Journal of Food Processing and Preservation, 44*, e14804. https://doi.org/10.1111/jfpp.14804

Djemam, N., Lassed, S., Gül, F., Altun, M., Monteiro, M., Menezes-Pinto, D., Benayache, S., Benayache, F., Zama, D., Demirtas, I. & Morato, M. (2020). Characterization of Ethyl Acetate and n-Butanol Extracts of *Cymbopogon schoenanthus* and *Helianthemum lippii* and their effect on the smooth muscle of the rat distal colon. *Journal of Ethnopharmacology, 252*, 112613. https://doi.org/ 10.1016/j.jep.2020.112613

Gunathilake, K.D.P.P., Ranaweera, K.K.D.S. & Rupasinghe, H.P. (2018). *In vitro* anti-inflammatory properties of selected green leafy vegetables. *Biomedicines, 6*(4), 107. https://doi.org/ 10.3390/ biomedicines6040107

Hamza, N., Berke, B., Umar, A., Cheze, C., Gin, H. & Moore, N. (2019). A review of algerian medicinal plants used in the treatment of diabetes. *Journal of Ethnopharmacology, 238,* 1-28. https://doi.org/ 10.1016/j.jep.2019.111841

Liu, X., Zhu, L., Tan, J., Zhou, X., Xiao, L., Yang, X. & Wang, B. (2014). Glucosidase inhibitory activity and antioxidant activity of flavonoid compound and triterpenoid compound from *Agrimonia pilosa* Ledeb. *BMC Complementary and Alternative Medicine, 14*(12), 1-10. https://doi.org/10.1186/ 1472-6882-14-12

Okur, M.E., Polat, D.C., Ozbek, H., Yilmaz, S., Yoltas, A. & Arslan, R. (2018). Evaluation of the

antidiabetic property of *capparis ovata* desf. var. *palaestina* zoh. extracts using *in vivo* and *in vitro* approaches. *Endocrine, Metabolic & Immune Disorders-Drug Targets (Formerly Current Drug Targets-Immune, Endocrine & Metabolic Disorders), 18*(5), 489-501. https://doi.org/10.2174/1871530318666180328110524.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*(9-10), 1231-1237. https://doi.org/10.1016/s0891-5849(98)00315-3

Rubio-Moraga, A., Argandoña, J., Mota, B., Pérez, J., Verde, A., Fajardo, J., Gómez-Navarro, J., Castillo-López, R., Ahrazem, O. & Gómez-Gómez, L. (2013). Screening for polyphenols, antioxidant and antimicrobial activities of extracts from eleven *Helianthemum* taxa (Cistaceae) used in folk medicine in South-Eastern Spain. *Journal of Ethnopharmacology, 148,* 287-296. https://doi.org/10.1016/j.jep.2013.04.028

Shinde, U., Phadke, A., Nair, A., Mungantiwar, A., Dikshit, V. & Saraf, M. (1999). Membrane stabilizing activity—a possible mechanism of action for the anti-inflammatory activity of *Cedrus deodara* wood oil. *Fitoterapia, 70*(3), 251-257. https://doi.org/10.1016/S0367-326X(99)00030-1

Sultana, B., Anwar, F. & Ashraf, M. (2009). Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules, 14,* 2167-2180. https://doi.org/10.3390/molecules14062167

Şam Gökşen, N. & Baldemir, A. (2016). Traditional usages of *Helianthemum* Mıller species. *Journal of Health Sciences, 25,* 49-52.

Terfassi, S., Dauvergne, X., Stephane Cerantola, S., Lemoine, C., Bensouici, C., Fadila, B., Christian M, Marchioni, E. & Benayache, S. (2021). First report on phytochemical investigation, antioxidant and antidiabetic activities of *Helianthemum getulum*. *Natural Product Research,* 1-8. https://doi.org/10.1080/14786419.2021.1928664

Yalcin, C.Ö., Yilmaz Sarialtin, S. & Cicek Polat, D. (2020). Quantification of phenolic and flavonoid contents and some biological activities of *Ornithogalum sigmoideum* Freyn & Sint. *Journal of Research in Pharmacy*, *24*(4), 487-496. https://doi.org/10.35333/jrp.2020.197