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Investigation of Antioxidant Effect of Origanum Hypericifolium Extracts in Different Solvents

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ABSTRACT

Origanum hypericifolium is one of the most widely distributed endemic species of the family Lamiaceae. Origanium hypericifolium contains mostly monoterpenes. Monoterpenes are volatile compounds with 10 carbons and are widely found in the plant kingdom. Monoterpenes, which are used as fragrances in perfumes and foodstuffs, have anti-fungal, anti-bacterial, anti-oxidant and anti-cancer effects.Various in vitro assays can be used to determine the antioxidant activity of *Origanium hypericifolium* extracts. In our study, total polyphenolic, total flavonoid content, iron reducing power, cupric ion reducing capacity and radical scavenging activity were evaluated to the antioxidant properties of various extracts of Origanum hypericifolium. TAS and TOS levels were also analyzed. The highest DPPH radical scavenging acitiviy value was 3.09±0.01 µg/mL in ethanol extract, Total polyphenol 144.00±1.89 µg GAE /g in ethanol extract, Total polyphenol 17. 08±0.85 µg CE /g in ethanol extract, FRAP 942.46±1.89 µg TE /g ethyl acetate and CUPRAC 2.54±0.01 mmol TE/g ethyl acetate extract.

Keywords: Antioxidants, Free radicals, Origanum hypericifolium.

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Introduction

Nowadays, studies on treatment methods with herbal medicines are increasing due to their low cost and easy availability. It is known that components isolated from plants are still being used in some types of cancer. For this reason, the search for new and effective herbal components continues.

Our country has a remarkably rich flora in terms of plant diversity. This richness is due to the fact that it is located at the intersection of three phytogeographic regions, it is a bridge between the flora of Southern Europe and South West Asia, and the centers of origin of many genera and sections are located in Anatolia. The flora of our country has a rich composition with 12.301 taxa. Of these taxa, 3963 are endemic and constitute 32.2% of our flora. In this respect, the possible pharmacological effects of many endemic species remain to be investigated. Origanum L. is a genus of the Lamiaceae family and includes 21 endemic species in Turkey, including Origanum hypericifolium O. Schwartz & P.H. Davis [1-3]. Members of this family are distributed mainly in the Mediterranean basin countries, but also in Australia, South West Asia and South America. Origanium hypericifolium includes the monoterpenes such as carvacrol, thymol and terpenes.

Members of the Lamiaceae family constitute a part of useful plants since ancient times. There are old records that the plants in this family are used in the treatment of various diseases [4-6]. O. hypericifolium, which is also found in Denizli region, is an endemic plant. It is a perennial O. hypericifolium, semi-shrub form, 30-105 cm tall and hairy. The leaves are petiolate, acute, greenish scaly, with black punctate glandular hairs [3]. O. hypericifolium is used as an herbal tea in the treatment of diabetes, as well as powdered and added as a spice to cheese and dishes [7]. The essential oil of the plant contains monoterpenes such as basic cymene, carvacrol, thymol and γ-terpinene [8,9]. Flavonoids, triterpenoids and monoterpenes are mostly distributed in the aerial parts of plants, especially in flowers and leaves, while phenolic acids and diterpenoids are mainly found in roots [10].

The purpose of the present work was to evaluate the antioxidant capacity of the extracts of Origanum hypericifolium, an Endemic herb, obtained in various solutions.

Methods

Preparation of Origanum Hypericifolium (EOH) Extracts in Different Solvents

C2 Muğla; Köyceğiz, Sandras Mountain, 1410 m, clearings of Pinus nigra (black pine forest), 16.09.2017, collected by M. Çiçek 2017-7-1 (Herb. M. Çiçek). While 55 g of Origanum hypericifolium was weighted, 1000 mL solvent was added to each one separately (Ethanol, ethyl acetate and n-hexane) and was sonicated at 45°C, 150 rpm

for 45 min. This process was repeated twice. The obtained samples were evaporated and the extracts were kept at -20oC until the experimental period [11].

Origanum Hypericifolium HPLC Analyses

Samples were eluted through 0.20 µm filters on a Perkin Elmer high pressure liquid chromatography (HPLC) and were analyzed on a Brownlee Analytical C18 (4.6 x 250mm, 5µm) colon (Perkin Elmer) with a DAD detector (Diode-Array Detector; Perkin Elmer Model Flexar, USA). Solvent A (orthophosphoric acid) and Solvent B (acetonitrile) were used. Samples were eluted from the column with a gradient of acetonitrile in 100%-57% water at 25 ºC and a flow rate of 0.8 mL/min (0-10 min 100% A, 8 min 100%-91% A, 10 min 91%-87% A, 8 min 87%-67% A, 14 min 67%-57% A) [12].

Determination of DPPH Radical Scavenging Activity

The method is based on the loss of the initial purple-violet colour of the DPPH (2,2-diphenyl-1-picrylhydrazyl] solution as a result of electron exchange with a hydrogen atom donating substance (antioxidant). 4 mg DPPH is dissolved in 10mL methanol. Thus, 100mL DPPH solution of 1 mM is prepared. The sample was mix with DPPH-radical (1:1) and was incubated in the dark for 50 min. At the end of the incubation period, absorbance is read at 517 nm (yellow colored reduced form). In this method, the change in absorbance of DPPH reacted with different sample concentrations is measured and the absorbance graph corresponding to the concentration is drawn. In the slope equation y=ax+b obtained from the graph, the amount of sample that halves the DPPH concentration is determined in µg/mL and expressed as the effective concentration 50 (IC50) value. The amount of antioxidant used to reduce the initial DPPH concentration by 50% is expressed as IC50 value [13,14].

Total Phenolic Substance Analysis

Total phenolic matter was analyzed by Folin-Ciocalteu assay. The method is based on the reduction of phosphotungstic acid. Folin-Ciocalteu reagent (10%) was added to samples diluted to different concentrations. This mixture was mixed with saturated sodium carbonate solution. The mixture was incubated in the dark for 30 min and then measured at 700 nm in a spectrophotometer. Total phenolic matter was expressed as gallic acid equiv (GAE) using the gallic acid calibration line [15].

Total Flavonoid Analysis

Total flavonoid content analysis performed according to Chang et al. The method is essentially the complexation of $AlCl_3$ by flavones. Samples were incubated with 80% ethanol, 10% $Al(NO_3)_3$ solution and 1 M KCH_3COO solution for 40 minutes at shade and absorbance was detected by spectrophotometer at 415 nm. Quercetin was used as standard [16,17].

Determination of FRAP Antioxidant Power

FRAP [Iron (III) reduction anti-oxidant capacity] determination was carried out according to the method developed by Benzie and Strain. According to this method, the main FRAP reagent, Phosphate Buffer and $FeCl_3$ solutions were mixed with the sample, incubated for 20 minutes in the shade and the absorbance was determined at 700 nm (Trolox was used as standard) [18].

Total Antioxidant Capacity Method Using Cu (II) as Oxidant (CUPRAC)

Cuprac ion reduction antioxidant capacity was determined according to the method of Arkan,T. CuCl2, neocuproin solutions were added to the sample and stored in the shade for 30 min and then the absorbance was measured at 450 nm in a UV-VIS [19].

Total Antioxidant Status (TAS) Assay

TAS was measured using Rel Assay, a novel assay method invented by Erel. In this technique, a hydroxyl radical is formed. This technique can be used to measure the anti-oxidative activity of the sample in response to strong free radical reactions initiated by the generated hydroxyl radical. The test has excellent sensitivity values of > 97% [20].

A2-A1=ΔA After taking the difference between absorbances, it is calculated according to equation 1 below.

$$x = \frac{\Delta A(\text{sample})}{\Delta A(\text{standart})} x20 \qquad (1)$$

Total Oxidant Status (TOS)

TOS determination was performed according to the method developed by Erel. In this method, R1 reagent (prepared by dissolving xylenol orange and NaCl in H_2SO_4 (pH=1.75) and adding glycerol), R2 reagent (prepared by dissolving ferrous ammonium sulphate hexahydrate and o-dianisidine dihydrochloride in H_2SO_4) were used. R1 and R2 reagents were added to the sample and incubated for 5 minutes at room temperature and the absorbance was measured at 530 nm [21].

A2-A1=ΔA After taking the difference between absorbances, it is calculated according to Equation 1.

Statistical Analysis

The analyses were conducted using SPSS version 22.0 (SPSS, Chicago, IL, USA). Measurement data are presented as mean ± standard deviation (SD). Statistical analyses involving multiple groups were conducted using either one-way ANOVA or the Kruskal-Wallis test. The mean IC50 values were calculated, and the averages were graphed using SigmaPlot version 12.0. A p-value of less than 0.05 was considered statistically significant.

Results

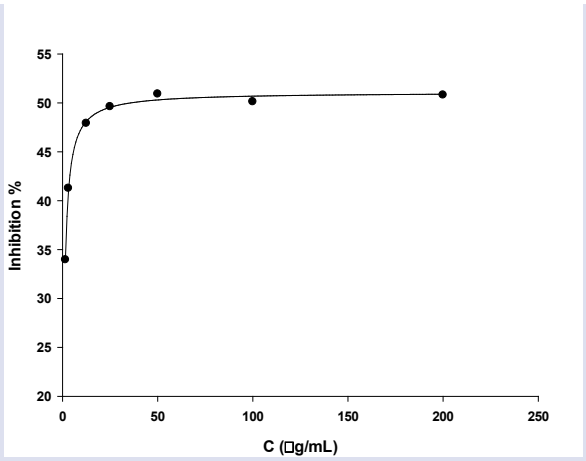
Since the antioxidant properties of Origanum Hypericifolium ethanol extract were found to be better, the content analysis was performed by HPLC. The chromatogram obtained is given as supplementary data 1 and the content is expressed in Table 1.

Table 1. Origanum Hypericifolium ethanol extracts of HPLC analysis

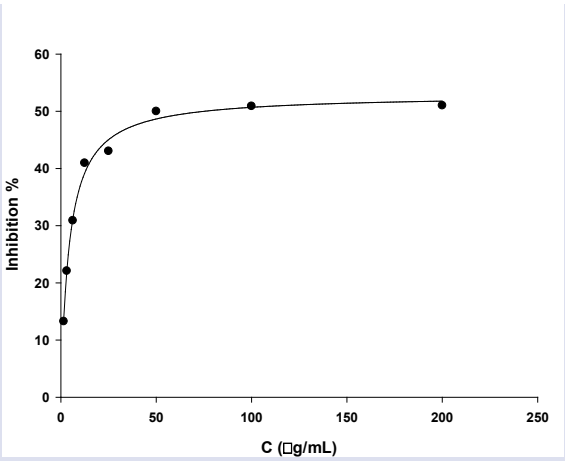
Sample No	Standart Name	Retion time	mg /g plant
1	Naringenin	14,6	90,4626
2	Rosmarinic asit	17,1	287,3615
3	Carvacrol	30	141,0442
4	Thymol	32,4	173,4577
5	Caffeic acid	33	204,5225
6	Coumaric acid	33,8	696,1726
7	Myricetin	34,7	539,198

As a result of HPLC analysis, the main components of carvacrol, thymol, caffeic acid, coumaric acid and myricetin were found abundantly in the ethanol extract (Supplementary 1).

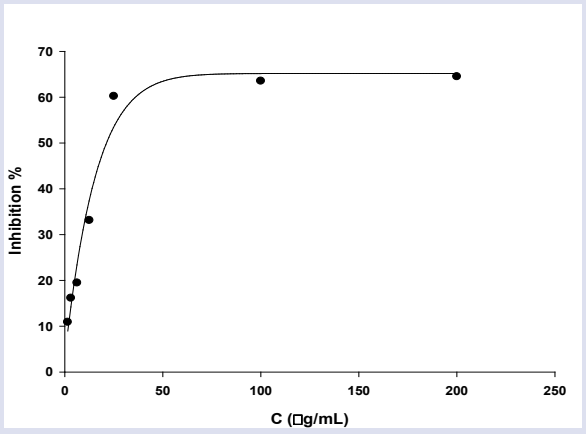
DPPH radical is used to determine the free radical scavenging activity of natural antioxidants. The free radical scavenging effects of *Origanum Hypericifolium* extracts of different solvents were determined by DPPH radical. DPPH solution is purple in colour. When the antioxidant interacts with a compound, its structure changes and forms a yellow colour and a new compound is formed. The rate of colour change is proportional to the concentration of the antioxidant. The concentration (C)-% inhibition graphs of the DPPH free radical scavenging activities of Origanum Hypericifolium extracts and ascorbic acid in the study are shown in Figure (1).



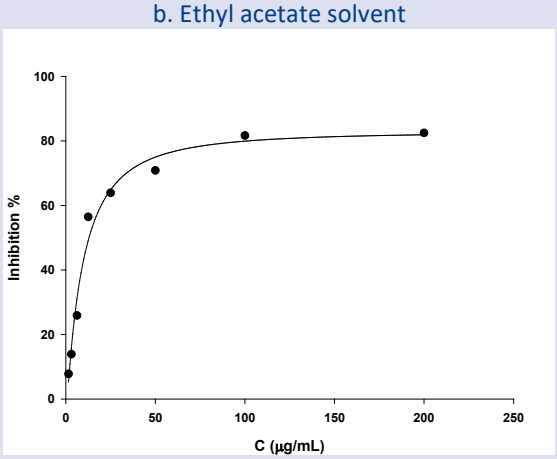
a.Ethanol solvent



b. Ethyl acetate solvent



c. n- hexane solvent



d. Ascorbic acid

Figure 1. DPPH graphs of Origanum Hypericifolium extract in different solvents (a. Ethanol solvent ; b. Ethyl acetate ; c. n- hexane ; d. Ascorbic acid)

The results of DPPH radical scavenging activity of *Origanum Hypericifolium* ethanol, ethyl acetate and n-hexane extracts are shown in Table 2.

Table 2. DPPH IC_{50} values

IC_{50}	Ethanol	Ethyl Acetate	n-Hexane	Ascorbic Acid
(µg/mL)	3.09±0.01	13.71±0.54	14.70±0.98	11.07±0.75

Total phenolic matter of the Origanum Hypericifolium was determined as gallic acid eq. Gallic acid was used as standard. Gallic acid calibration graph was found as y=0.0370+0.0006x (R2=0.9993).

The results of the total phenolic content of *Origanum Hypericifolium* ethanol, ethyl acetate and n-hexane extracts are shown in Table 3.

Table 3. Total Phenolic Substances

	Ethanol	Ethyl Acetate	n-Hexane
(µg GAE /g)	144.00±1.89	138.94±1.78	30.50±1.08

Total flavonoid content of the extracts of Origanum hypericifolium in different solvents was calculated as quercetin equivalent. Quercetin was used as standard. The equation y=0.0433+0.0648x (R2=0.9999) was found from the quercetin standard graph.

The results of total flavonoid content of Origanum Hypericifolium ethanol, ethyl acetate and n-hexane extracts are shown in Table 4.

Table 4. Flavonoid values

	Ethanol	Ethyl Acetate	n-Hexane
(µg CE /g)	17.08±0.85	15.69±0.66	7.09±0.47

The amount of FRAP antioxidant power was calculated as trolox equivalent. Trolox was used as the standard. The standard graph of trolox was found as y=0.1511+0.0005x (R2=0.8772).

The results of FRAP antioxidant power of Origanum Hypericifolium ethanol, ethyl acetate and n-hexane extracts are shown in Table 5.

Table 5. FRAP values

	Ethanol	Ethyl Acetate	n-Hexane
(µg TE/g)	683.11±1.76	942.46±1.89	200.46±1.01

Calculation of total antioxidant capacity value equivalent to trolox in solid samples is done by using Equation 2 given below. When the total antioxidant capacity value is to be calculated as the equivalent of a different antioxidant, the molar absorption coefficient of the relevant antioxidant compound should be used in the formula.

$$TAC(mmol\frac{TR}{g} - sample = \frac{A}{\epsilon}\times\frac{Vt}{Vs}\times DF\times\frac{Ve}{m} \quad (2)$$

A: Absorbance, Ɛ: Molar absorption coefficient (16700 L mol-1.cm-1),*Vt*: Total volüme, *Vs*: Sample volüme, DF: Dilution factor ,*Ve*: Volume of prepared extract, m: Weight of sample in the extraction process (g)

Cu (II) results of *Origanum Hypericifolium* ethanol, ethyl acetate and n-hexane extracts as oxidant are shown in Table 6.

Table 6. CUPRAC values

(mmol TE/g)	Ethanol	Ethyl Acetate	n-Hexane
	1.64±0,09	2.54±0.01	1.35±0.06

Total antioxidant status and Total oxidant status results of *Origanum Hypericifolium* ethanol, ethyl acetate and n-hexane extracts are shown in Figure 2.

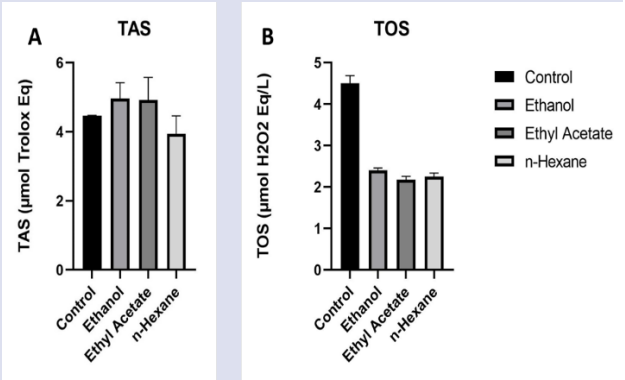


Figure 2. Total antioxidant status (A) and Total oxidant status (B) results of *Origanum Hypericifolium* Extracts

Table 7. The Antioxidant Values of Origanum hypericifolium Extracts in Various Solvents

	Ethanol	Ethyl Acetate	n-Hexane	Ascorbic acid	Control	p-value
DPPH (µg/mL)	3.09±0.01	13.71±0.54	14.70±0.98	11.07±0.75	-	p<0.0000
Polifenol (µg GAE /g)	144.00±1.89	138.94±1.78	30.50±1.08	-	-	p<0.0000
Flavonoid(µg CE /g)	17.08±0.85	15.69±0.66	7.09±0.47	-	-	p<0.0000
FRAP (µg TE /g)	683.11±1.76	942.46±1.89	200.46±1.01	-	-	p<0.0000
CUPRAC (mmolTE/g)	1.64±0,09	2.54±0.01	1.35±0.06	-	-	p<0.0000
TAS (µmol Trolox Eq)	5.25±0.19	5.15±0.21	3.99±0.13	-	4.22±0.02	0.0000152
TOS(µmol H_2O_2 Eq/L)	2.35±0.01	2.13±0.06	2.28±0.07	-	4.58±0.34	0.000000380

* In statistical analysis, a p-value of less than 0.05 is considered significant.

Dissuccions

Medicinal plants have been used since ancient times for the treatment of many diseases. Today, at least 132 different active substances derived from these plants are currently in use and recognized as important medicines [22]. In this context, new medicinal plants are continuously researched and contribute to the literature and new treatments.

There are some literature studies investigating the anatomical, morphological, cytotaxonomic, palynological, biosystematic, chorological and chemical characteristics of Origanum genera in Turkey [23]. Origanium hypericifolium essential oil contains monoterpenes such as p-cymene, carvacrol, thymol and γ-terpenes. In our content analysis (by HPLC), carvacrol, thymol, caffeic acid, coumaric acid and myricetin were abundant in the ethanol

extract of Origanium hypericifolium. Carvacrol and thymol are monoterpenes; while caffeic acid is a phenolic group; coumaric acid and myricetin are in the polyphenol group. Monoterpenes have antifungal, antibacterial, antioxidant, anticancer, antispasmodic, hypotensive and vasorelaxant effects [8,9].

Antioxidant is the general name for a group of compounds that stop oxidation reactions caused by free radicals. These compounds neutralize free radicals, preventing the body from being affected by them and allowing it to regenerate itself. Determination of the total amount of phenolic substances in foods is important in terms of giving an idea about the hydroxyl groups that provide antioxidant activity. Generally, there is a good linear correlation between total phenol content and antioxidant activity. Various in vitro assays can be used to determine the antioxidant activity of natural product extracts [24]. Oxygen Radical Absorbance Capacity (ORAC) Method, Total Radical Scavenger Parameter (TRAP) Method, Crocin Whitening Method, Total Oxyradical Quenching Capacity (TOSC) Method, Trolox Equivalent Antioxidant Capacity (TEAC or ABTS) Method, DPPH Radical Scavenging Capacity Method, CUPRAC Method, Iron (III) Ion Reducing Antioxidant Power (FRAP) Method, Folin-Ciocalteu Reagent (FCR) and Total Phenolic Method [25]. In our study, total polyphenolic, total flavonoid content, iron reducing power, cupric ion reducing capacity and radical scavenging activity were evaluated to the antioxidant properties of various extracts of Origanum hypericifolium. TAS and TOS levels were also analyzed.

In our study, the DPPH radical sacvenging activity of Origanum Hypericifolium ethanol extract was the strongest. The IC50 value of DPPH radical was 3.09±0.01µg/ml for ethanol, 13.71±0.54 for ethyl acetate, 14.70±0.98 for n-hexane and 11.07±0.75µg/ml for ascorbic acid as standard. The IC50 value of DPPH radical of Origanum Hypericifolium essential oil collected from the dry limestone slopes of Korkuteli district, Antalya was determined as 4.88 mg/ml. Hossain, M. et al. found the IC50 value of DPPH radical in Methanol extracts of Origanum species to be 20.10 µg/g [26]. Sökmen M. et al. determined the IC50 values of DPPH radical in methanol, dichloromethane (DCM) extracts of Origanum species as 18.0 ± 2.0, 49.5 ± 3.5 µg /mg, respectively [27]. Roby, M. et al. reported that the IC50 values of DPPH radical in methanol, ethanol, diethyl ether, hexane extracts of Origanum species were 11.00±0.1, 13.00±0.1, 13.00±0.1, 13.00±0.2, 22.00±0.5 mg GAE/g, respectively [28].

In our study, the total polyphenol content of ethanol extract of Origanum Hypericifolium was 144.00±1.89, ethyl acetate 138.94±1.78, 30.50±1.08 n-hexane µg GAE / g. Celik A. et al. reported 1.2480±0.03 mmol GAE/L total polyphenolic content in Origanum Hypericifolium essential oil collected from Sandras mountain [29]. Fakir H. et al. reported 104.928±2.389 mg GAE/g total polyphenolic content in Origanum Hypericifolium essential oil collected from Denizli Honaz Mountain [30]. Sökmen M. et al. found total polyphenolic content as 151.5± 12.0, 154.0 ± 9.6 and 1.9 ± 0.4 µg /mg in methanol, dichloromethane and hexane extracts of Origanum species, respectively [27]. Roby et al. reported 24.66±3.90, 14.80±200, 10.30±2.08, 4.21±2.65 mg GAE/g of total polyphenolic content in methanol, ethanol, diethyl ether, hexane extracts of Origanum species [28]. Cervato, G. et al. found total polyphenolic content of 5±0.1 µg GAE /g in methanol extracts of Organum vulgare [31]. Hossain, M. et al. determined total polyphenolic content of 11.87 µg/g in Methanol extracts of Origanum species [26].

Our study, amount of total flavonoid of extracts was 17.08±0.85, 15.69±0.66 and 7.09±0.47 µg CE/g in ethanol, ethyl acetate and n-hexane, respectively. In a study by Özkan, G. et al. total flavonoid content in hexane and ethyl acetate extracts were 716.86±1.17 / 1291.69±1.67 µg CE/g, respectively [32]. In the study by Kaurinovic, B. et al. on different species of Origanum, the total flavonoid content in the extracts prepared in ethyl acetate and chloroform solvents was found to be 9.37 ± 0.06 / 13.12 ± 0.05 mg / g, respectively [33].

In our study, iron reducing power determination was 683.11±1.76, 942.46±1.89 and 200.46±1.01 µg TE /g in ethanol, ethyl acetate and n-hexane, respectively. According to the study conducted by Baddal, M. et al. iron reducing power determinations in hexane, dichloromethane, chloroform, acetone, methanol extracts were 0.117 ± 0.010, 1.237 ± 0.033, 0.343 ± 0.008, 1.272 ± 0.001, 1.583 ± 0.110 mg CE/mg respectively [34]. Sarikurkcu, C. et al. found iron reducing power 17.18 ± 0.27 mg /g [35].

In our study, CUPRAC ion reduction capacity was 1.64±0.09, ethyl acetate 2.54±0.01, n-hexane 1.35±0.06 mmol TE/g for ethanol. In a study by Yılmaz, H. et al. the CUPRAC ion reducing capacity of ethanol extracts was found to be 0.9 mmol /g [36]. According to another study by Sarikurkcu, C. et al. the CUPRAC ion reducing capacity was found to be 46.64 ± 0.37 mg /g [35].

The total antioxidant capacity of Origanum Hypericifolium was studied and 4.96±0.46, 4.92±0.65, 4.94±0.72 mmol TE/L for ethanol, ethyl acetate, n-hexane were found. Celik A. et al. reported a total antioxidant content of 1.5358±0.06 mmol GAE/L in Origanum Hypericifolium essential oil collected from Sandras mountain [29]. Fakir H. et al. found the total antioxidant content of Origanum Hypericifolium essential oil collected from Denizli Honaz Mountain as 9.979±0.309 mg GAE/g [30].

According to the results of the total oxidant capacity of Origanum Hypericifolium extract obtained in different solvents, it was found as 2.40±0.06, 2.18±0.08, 2.25±0.09 µmol H2O2 / L for ethanol, ethyl acetate, n-hexane. According to the study of Celik A. et al. total oxidant capacity was found as 95±2 µmol H_2O_2 / L [29].

When the literature is examined, it is observed that the results of total polyphenol, flavonoid, iron reducing power (FRAP), radical scavenging activity (DPPH), CUPRAC, TAS and TOS analyzes are compatible with the literature in our study with different extracts of Origanum

Hypericifolium. Further studies are needed to determine the effects of Origanum Hypericifolium.

Conclusions

About 50% of medicines used for therapeutic purposes are herbal. Although plants have many active molecules, there are many factors in secondary metabolites whose effectiveness has not yet been elucidated. Thus, due to the need for effective bioactive agents, research on phytochemical and bioactivity properties of plants based on ethnobotanical approach has gained momentum [37]. Although metabolites in plant products are generally of low risk, some pure compounds may be toxic. Therefore, in vitro and in vivo screening tests of herbal products are important to demonstrate their toxic effects [38].

In conclusion, Origanum Hypericifolium was observed to have high antioxidant properties. Clarification and characterization of the compounds active in herbs and a greater understanding of the respective preservative mechanisms are essential for their possible use in pharmaceuticals industry.

Conflicts of interests

No conflicts of interest have been declared by the authors.

References

[1] Başer K.H.C.,(2002). The Turkish Origanum Species, In:Oregano, The Genera Origanum and Lippia, Ed.: S.E.Kintzios, Taylor and Francis, UK.

[2] Başer K.H.C., Tümen G., Özek T. ve Kürkçüoğlu M., Composition of the Essential Oil of Origanum sipyleum of Turkish Origin, *Journal of Essential Oil Research*, 4(1) (1992)139-142.

[3] Davis P.H., (1982). Flora of Turkey and the East Aegean Islands, vol: 7. Edinburg University Press, Edinburgh.

[4] Baytop T. (1999). Türkiye'de bitkiler ile tedavi (Geçmişte ve Bugün). İstanbul Üniversitesi Yayınları, İstanbul, Türkiye, 520s.

[5] Baytop T., (1994).Türkçe Bitki Adları Sözlüğü, Atatürk Kültür, Dil ve Tarih Yüksek Kurumu, Türk Dil Kurumu Yayınları: 578, s.33, 206, Ankara.

[6] Kütükcü Z.M. (2016). Deneysel diyabetik dişi sıçan genital sisteminde Origanum Hyperıcıfolıum Esansiyel yağının etkilerinin ince yapı düzeyinde araştırılması (Master's thesis, Pamukkale Üniversitesi Sağlık Bilimleri Enstitüsü).

[7] Sonmez C., Effect of different harvest times on some yield and essential oil characteristics in Origanum onites L. *Turkish Journal Of Field Crops*, *24*(1) (2019)106-110.

[8] Marino M., Bersani C., Comi G., Impedance measurements to study the antimicrobial activity of essential oils from Lamiaceae and Compositae. *International journal of food microbiology*, *67*(3) (2001) 187-195.

[9] Özcan M, Erkmen O,, Antimicrobial activity of the essential oils of Turkish plant spices, *European Food Research and Technology,* 212(6) (2001) 658-660.

[10] Topçu G., Bioactive triterpenoids from Salvia species. *J Nat Prod,* 69 (2006) 482–487.

[11] Tutar U., Hepokur C., Misir S., Hepokur A.I., DUMAN F., Antimicrobial, Antioxidant, Cytotoxic and Wound Healing Effects of Thymbra sintenisii Extract. *Indian Journal of Pharmaceutical Sciences*, *80*(5) (2018).

[12] Gök H. N., Luca S. V., Ay S.T., Komsta Ł., Salmas R. E., Orhan I. E.,Skalicka-Woźniak K., Profiling the annual change of the neurobiological and antioxidant effects of five Origanum species in correlation with their phytochemical composition. *Food Chemistry*, *368* (2022) 130775.

[13] Cuendet M., Potterat O., Salvi A., Testa B.,Hostettmann K., A stilbene and dihydrochalcones with radical scavenging activities from Loiseleuria procumbens. *Phytochemistry*, *54*(8) (2000) 871-874.

[14] Berköz M., Yalın S., Normal ve preeklamptik gebelerde lipid peroksidasyonu ve antioksidan aktivite. *ADÜ Tıp Fakültesi Dergisi* 10 (2009) 52-58.

[15] Dinçer Y., Akçay T., DNA Hasarı. *Türk Biyokimya Dergisi*,25(2) (2000)73-79.

[16] Lima C.F., Fernandes-Ferreira M., Pereira-Wilson C. Phenolic compounds protect HepG2 cells from oxidative damage: Relevance of glutathione levels. *Life Sciences*, 79 (2006)2056-2068.

[17] Chang C.C., Yang M.H., Wen H.M., Chern J.C., Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Anal,* 10(3) (2002)178-82

[18] Yu L., Haley S., Perret J., Harris M., Wilson J., Qıan M., Free radical scavenging properties of wheat extracts. *J Agric Food Chem,* 50(6)(2002)1619-24.

[19] Arkan T. (2011). Daphne oleoides subsp. oleoides ve Daphne sericea'nın farklı çözücülerle antioksidan özellikleri (Doctoral dissertation, Selçuk Üniversitesi Fen Bilimleri Enstitüsü).

[20] Erel O., A novel automated direct measurement method fortotal antioxidant capacity using a new generation, morestable ABTS radical cation. *Clin Biochem,* 37(2004) 277–285.

[21] Erel O.,A new automated colorimetric method for measuring total oxidant status. *Clin Biochem,* 38 (2005)1103–1111

[22] Emine F., (2013). Isparta ve Afyon illerinde tıbbi ve aromatik amaçlı kullanılan bitkilerin satılma, toplanma ve kullanılma durumları. Doktora tezi, Ankara Üniversitesi Fen Bilimleri Enstitüsü.

[23] Azize T.,(2010). Türkiye'de yetişen Orıganum L. (Labıatae) cinsi üzerinde epidermal incelemeler. Yüksek lisans tezi. İnönü Üniversitesi Sağlık Bilimler Enstitüsü.

[24] Jin D., Mumper R.J., Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules,* 15(10) (2010)7313-52.

[25] Okan O. T., Varlıbaş H., Mehmet Ö.Z., Deniz İ., Antioksidan analiz yöntemleri ve doğu Karadeniz bölgesinde antioksidan kaynağı olarak kullanılabilecek odun dışı bazı bitkisel ürünler. Kastamonu Üniversitesi Orman Fakültesi Dergisi, 13(1) (2013) 48-59.

[26] Hossain M.B., Brunton N.P., Patras A., Tiwari B., O'donnell C.P., Martin-Diana A.B., Barry-Ryan C., Optimization of ultrasound assisted extraction of antioxidant compounds from marjoram (Origanum majorana L.) using response surface methodology. *Ultrasonics sonochemistry*, 19(3) (2012)582-590.

[27] Sökmen M., Serkedjieva J., Daferera D., Gulluce M., Polissiou M., Tepe B., Sokmen A., from herbal parts and callus cultures of Origanum acutidens. *Journal of agricultural and food chemistry*, 52(11)(2004) 3309-3312.

[28] Roby M. H. H., Sarhan M. A., Selim K.A.H., Khalel K.I. Evaluation of antioxidant activity, total phenols and phenolic compounds in thyme (Thymus vulgaris L.), sage (Salvia officinalis L.), and marjoram (Origanum majorana L.) extracts. *Industrial Crops and Products*, 43 (2013) 827-831.

[29] Celik A., Nur Herken E., Arslan İ., Zafer Özel M.,Mercan N. Screening of the constituents, antimicrobial and antioxidant activity of endemic Origanum hypericifolium O. Schwartz & PH Davis. *Natural product research*, 24(16) (2010)1568-1577.

[30] Fakir H., Us A.A., Sagdic M.,Tornuk F.,Essential Oil Composition, Antimicrobial and Bioactive Properties of Origanuim hypericifolium, An Endemic Plant Species grown in Turkey. *Research Journal of Biotechnology*,10, 11(2015).

[31] Cervato G., Carabelli M., Gervasio S., Cittera A., Cazzola R.,Cestaro B., Antioxbdant properties of oregano (Origanum vulgare) leaf extracts. *Journal of Food Biochemistry*, 24(6) (2000). 453-465.

[32] Özkan G. (2007). Türkiye'de Lamiaceae (Labiatae) familyasına ait baharat veya çeşni olarak kullanılan bazı bitkilerin fenolik bileşenleri ile antioksidan ve antimikrobiyal etkilerinin belirlenmesi (Doctoral dissertation, Selçuk Üniversitesi Fen Bilimleri Enstitüsü).

[33] Kaurinovic B., Popovic M., Vlaisavljevic S., Trivic S. Antioxidant capacity of Ocimum basilicum L. and Origanum vulgare L. extracts. *Molecules,* 16(9) (2011) 7401-7414.

[34] Baddal M.K. (2010). Origanum Minutiflorum bitkisinin antioksidan özelliklerinin incelenmesi (Doctoral dissertation, SDÜ Fen Bilimleri Enstitüsü).

[35] Sarikurkcu C., Zengin G., Oskay M., Uysal S., Ceylan R., Aktumsek A., Composition, antioxidant, antimicrobial and enzyme inhibition activities of two Origanum vulgare subspecies (subsp. vulgare and subsp. hirtum) essential oils. *Industrial Crops and Products,* 70 (2015)178-184.

[36] Yılmaz H., Çarıkçı S., Kılıç T., Dirmenci T., Arabacı T., Gören A.C., Screening of chemical composition, antioxidant and anticholinesterase activity of section Brevifilamentum of Origanum (L.) species. *Records of Natural Products,* 11(5) (2017).

[37] Elhardallou S.B., Cytotoxicity and biological activity of selected Sudanese medicinal plants. *Research Journal of Medicinal Plant*, 5(3) (2011) 201-29.

[38] Taraphdar A.K., Roy M., Bhattacharya R.K. Natural products as inducers of apoptosis: Implication for cancer therapy and prevention, *Curr Sci*, 80 (2001) 1387-1396.