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The phenolic content, antioxidant and cytotoxic activities of *Origanum sipyleum* from Turkey

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Abstract: O*riganum sipyleum* is a medicinal plant species belonging to the genus *Origanum* and traditionally vegetative parts of this plant have been used as medicinal tea or food additives. The current study has been designed to examine the antioxidant, cytotoxic activities and total phenolic, flavonoid and tannin contents as well as the chemical composition of the ethanol extract of *O. sipyleum*. The antioxidant capacity of *O. sipyleum* was determined using six complementary methods, β-carotene/linoleic acid and phosphomolybdenum test systems, radical scavenging (ABTS and DPPH), metal chelating and reducing power assays. The phenolics were identified using HPLC. A brine shrimp *(Artemia salina L.)* lethality test was used for determining cytotoxic activity. The ethanol extract exhibited high DPPH free radical scavenging (DPPH, IC_{50}:102.75 µg/mL), ABTS radical scavenging (ABTS, IC_{50}:88.64 µg/mL), metal chelating (20.68 %) and reducing power capacity (0.51 mg/mL). The antioxidant activities of the *O. sipyleum* with β-carotene/Linoleic acid and phosphomolybdenum were calculated as 85.59 % and 62.95 µg/mg respectively. The phenolic contents of the ethanol extract were evaluated using HPLC and determined major phenolics: caffeic acid, epicatechin and 2,5 dihydroxybenzoic. Furthermore, to gether with cytotoxic activity (LC_{50}, 327.414 µg/mL) *O. sipyleum* is also rich in total phenolic, flavonoid and tannin contents were 203.57 ± 4.62 mgGAE/g, 46.98 ± 0.34 mgQE/g and 34.55 ± 0.56 mgCE/g respectively. These results could provide addition information for the potential use of this medicinal plant as a food ingredient and as a natural antioxidant in the diet, as well as for the pharmaceutical industry.

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1. INTRODUCTION

Free radicals are unstable molecules that have been shown to react with various other materials to form new compounds that contain high levels of toxicity. Free radicals are able to induce cellular damage, oxidize protein, lipid and thereby can cause diseases [1, 2]. Antioxidants are substances that may inhibit these illnesses and due to these beneficial effects. Food and medicinal products are routinely enriched with synthetic antioxidants (BHT and

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BHA). Conversely, there is evidence of manufactured antioxidants being potentially toxic. Therefore, there is great interest in the isolation of the safer and effective antioxidants that are naturally present in plants [3-5].

Plants possess active natural compounds that differ widely in terms of biological properties and can be used in various fields, such as food ingredients, as well as medicinal and pharmacological applications. For this reason, there has been increased interest in research on the isolation and identification of these compounds [6,7]. Many plants belonging to the Lamiaceae family contain chemical compounds with antioxidant activity [6]. Among the Lamiaceae members, *Origanum* species are among the important and popular plants. They are used as an anti-diabetic, digestive, diuretic, and for respiratory problems [8]. *Origanum sipyleum* is one of the endemic species of the *Origanum* genus and used as a medicinal tea or as a food additive [9].

Previous studies on antioxidant capacities of *Origanum* species were conducted [10-13]. There were also few studies dealing with the biological activities of *O. sipyleum* [8, 9, 14], but there were no comprehensive report that has studied the phenolic composition, antioxidant and cytotoxic activities of ethanol extract of *O. sipyleum*. Therefore, more research is required on the biological activities of this aromatic and medicinal plant. With these points in mind, the objectives of the present study are to evaluate the antioxidant capacities, the cytotoxic activity, the total phenolic, flavonoid and tannin contents as well as the chemical composition of the ethanol extracts of *O. sipyleum*.

2. MATERIALS and METHODS

2.1. Plant materials and preparation of plant extracts

O. sipyleum. was collected in June 2017 from above the Çamlık forest, on the old Denizli-Kızılcabölük road, in mixed *'Pinus brutia-Quercus coccifera'* forest clearings, Denizli, Turkey The plant materials were identified and stored with voucher specimens (*O. sipyleum*; Herbarium No: No: 2017-4-92) at the private herbarium of Dr. Mehmet Cicek, a plant taxonomist from the Pamukkale University, Denizli, Turkey. The stem, flowers and leaves of *O. sipyleum* were air-dried and milled. The extractions were performed by mixing the sample (20 g) with 200 mL of ethanol and shaking at 50 ^{o}C for 6 h in a temperature controlled shaker. The extracts were filtered twice with filter paper and evaporated using a rotary evaporator under vacuum at 40-50^{o} C. The samples were lyophilized and kept at -20 ^{o}C until tested. The assays were carried out in three sample replications and values were represented as the average of three replicates.

2.2. Evaluation of DPPH free radical and ABTS radical cation scavenging activity

ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) was used for determining radical cation scavenging activity according to the method described by Shalaby and Shanab [15]. ABTS radical cation was prepared by reacting 7mM ABTS stock solution with 2.45 mM potassium persulfate and the mixture was held for ~16 h in a dark room. Prior to the assay, the ABTS solution was diluted with ethanol to an absorbance of 0.700±0.05 at 734 nm. A total of 900 µL ABTS solution was mixed with 100µL of the extracts. The mixture was incubated at room temperature for 15 min. and then the absorbance of this mixture was measured. The results were assessed as IC_{50} values (concentration in µg/mL that cause 50% inhibition of the ABTS radicals).

The DPPH (2,2-Diphenyl-1-picryl hydrazyl radical) free radical was used for determining the free radical scavenging activity according to the procedure of Meriga et al. [16]. The different concentrations of the extracts (1 mL) were mixed with methanolic DPPH solution (4 mL). After 30 minutes, the absorbance of the extracts was recorded at 517 nm. These outcomes

were calculated as IC_{50} values, that is the strength of the sample required to scavenge 50% of DPPH radicals.

2.3. Determination of total antioxidant capacity by β-carotene/linoleic acid and Phosphomolybdenum assay

The antioxidant capacity was evaluated using the method of Amin and Tan [17]. β-carotene (2 mg) was dissolved in chloroform (10 mL) to prepare the stock solution and Linoleic acid (20 µL) and 200 µL of 100% Tween 20 was added for one milliliter of the solution. The chloroform was removed using a rotary evaporator and then the remaining residue was added to 100 mL of dH_2O. This emulsion (24 mL) was mixed with the extracts (1 mg/mL). A spectrophotometer was immediately used to measure the initial absorbances at 470 nm. The reaction mixture was incubated for 2 hours at 50° C. Following this the measurement of the absorbance of this mixture was repeated, and a synthetic antioxidant (BHT, Butylated hydroxytoluene) was applied as the positive control.

The antioxidant capacity of *O. sipyleum* extract was also determined using the phosphomolybdenum method according to that of Prieto et al. [18]. The sample preparation and procedure followed that given by Kaska et al. [19]. The antioxidant activity of the extracts was calculated using the ascorbic acid graph.

2.4. Measurements of Ferric ion reducing power activity

The reducing power of the *O. sipyleum* was estimated using the method described by Oyaizu [20] with slight modifications. Different concentrations of the samples (1 mL) were combined with 0.2-M phosphate buffer (1 mL) and 1% potassium ferricyanide (1 mL). The mixture was kept at 50°C for 20 min. Trichloroacetic acid (10%) was added to reaction mixture. The aliquot of the upper layer (1.5 mL) was combined with the same volume of the ddH_2O and 0.1% ferric chloride. After 10 min the absorbance was read, at 700 nm.

2.5. Measurements of Metal chelating capacity

The metal chelating assay was conducted using the method of Karpagasundari and Kulothungan [21] with slight modifications. The plant sample and 3.2 mL of ddH_2O was mixed with 2 mM $FeCl_2$ (0.1 mL) solution. After 30 s, ferrozine (5 mM) was added. By adding ferrozine, the reaction was activated. After approximately 10 min at room temperature, the absorbance of the solutions was read at 562 nm. The synthetic metal chelator (EDTA) was applied as the positive control. The metal chelating activity was calculated in the following way:

$$\text{Chelating ability (\%)} = [(A_{co} - A_{samp}) / A_{co}] \times 100,$$

(A_{co} : absorbance of the control and A_{samp} : absorbance of the extract or positive control, EDTA.)

2.6. Determination of total phenolic, flavonoid and tannin contents

The total phenolic content was ascertained according to the Folin-Ciocalteu method [22], the total flavonoid content of the extracts was determined using the method of Arvouet-Grand et al. [23] and the Tannin content was investigated using the vanillin-HCL method [24]. The method of Kaska et al. [19] provided the sample preparation and procedure for determining the total phenolic, flavonoid and tannin content. The outcomes were shown as the equivalents of Gallic acid (mgGAE/g), quercetin (mgQEs/g) and catechin (mgCEs/g) for phenolic, flavonoid and tannin content respectively.

2.7. Quantification of phenolic compounds by HPLC

For the determination of the phenolic compound reversed-phase high performance liquid chromatography (RP-HPLC, Shimadzu Scientific Instruments) was used. The phenolic composition of the ethanol extract of *O. sipyleum* was determined according to the method of Caponio et al. [25] with slight modifications. The procedure followed was given by Kaska et al. [19]. Gallic, 3,4 dihydroxybenzoic, 4-hydroxybenzoic, 2,5 dihydroxybenzoic, chlorogenic, vanillic, caffeic, *p*-coumaric, ferulic, cinnamic acid and quercetin, epicatechin, rutin were used as standards and quantitative analysis was made by comparing these standards. The results were expressed as µg/g of each compound from the total phenolic compounds.

2.8. Cytotoxic bioassay

The possible cytotoxic activity of *O. sipyleum*, was evaluated using the Brine shrimp lethality bioassay [26]. The brine shrimps (*Artemia salina*) were hatched using *A. salina* eggs in a beher-beaker (1 L), filled with air-bubbled sterile artificial seawater (prepared using sea salt 38 g/L) and left to incubate under artificial light for 24-48 h at 28 ºC. The tubes containing ten nauplii, different concentration of extracts (1000, 500, 100, 50 and 10ppm) and brine solution as well as the control tubes were maintained under artificial light for 24 h at 28 ºC. The experiments were conducted in a set of three tubes per concentration and the controls. For each concentration of the extracts and the controls, the number of dead shrimps were counted and recorded using an overhead projector. The larvae were regarded as dead if no activity of the appendage was seen within 10 sec. The EPA Probit Analysis Program was used for data analysis.

2.9. Statistical analysis

The standard and the different groups were compared via t-test by using MINITAB.

3. RESULTS and DISCUSSION

An evaluation of the antioxidant capacity of plants cannot be conducted by a single standard method due to the complex structure of the compound to be analyzed. Consequently, a single assay could not accurately reflect the antioxidant capacities of the plants [27]. For this reason, several antioxidant methods (DPPH, ABTS, phosphomolybdenum, metal chelating activity etc.) were applied to evaluate the true antioxidant potential of the *O. sipyleum*.

3.1. Radical scavenging capacity (DPPH and ABTS)

A well-known mechanism for ascertaining the antioxidant activity of plants is through ABTS radical scavenging capability hydrogen donating [28]. The results of the radical scavenging capability were calculated to be a concentration, 50% of which was scavenged by ABTS (IC_{50}). The low IC_{50} value shows the high radical scavenging property. The ABTS radical scavenging capacity of extracts from *O. sipyleum* is presented in Table 1 and there were statistically differences among the radical scavenging activity of the ethanol extract of *O. sipyleum* and BHT ($t=31.47$, $df=11$, $p<0.001$).

The results of the DPPH free radical scavenging activity presented in Table 1 and the findings indicate that the ethanol extract of *O. sipyleum* exhibits radical scavenging activity. The DPPH radical scavenging activities for the ethanol extract determined in this study were higher than in methanol, water and acetone extracts and lower than in the ethanol extract from *O. sipyleum* as reported by Nakipoglu et al. [8]. The phenolic content may be attributed to the DPPH radical scavenging activity of *O. sipyleum*. Oxidative damage caused to cells by free-radicals may potentially be mitigated by these phenolic compounds [29]. As shown in the present study, the ethanol extract of *O. sipyleum* has potent radical scavenging activities and findings reveal that this extract could serve as a strong radical scavenger, and due to this

property it could be possible to use *O. sipyleum* plants in pharmacological applications as radical inhibitors or scavengers.

Table 1. Antioxidant properties of O.*sipyleum*

Sample	DPPH (IC_{50}, µg/mL)	ABTS (IC_{50}, µg/mL)	β-carotene/linoleic acid (%)	Phosphomolybdenum (µg/mg)	Power reducing activity (mg/mL)	Metal chelating activity (%)
Ethanol	102.75 ± 1.6 a	88.64 ± 2.1 a	85.59 ± 1.2 b	62.95 ± 1.36	0.51 ± 0.08	20.68 ± 2.6 b
BHT	31.76 ± 1.7 b	12.89 ± 1.2 b	93.46 ± 0.3 a	nt	nt	nt
EDTA	nt	nt	nt	nt	nt	76.41 ± 0.2 a

BHT: Standard antioxidant, nt: not tested

*Values are mean of three replicate determinations (n=3) ± standard error. Mean values followed by different letters in a column are significantly different ($p<0.05$).

3.2. Antioxidant activity (β-carotene/linoleic acid and Phosphomolybdenum method)

The one of the main causes of food quality deterioration is lipid peroxidation. A antioxidants have the capability to delay the development of toxic oxidation, prolong the storage stability of foods and maintain nutritional quality and for this reason they are used in lipid containing foods [30, 31]. Nowadays, because of their antioxidant content numerous plants are regularly used as sources of nutritional additives to inhibit lipid peroxidation. Medicinal Lamiaceae plants with high antioxidant capacities are known to be efficient in delaying the process of lipid peroxidation in fatty foods [32]. In the present study, β-Carotene assay was used to evaluate the capability of plants regarding the inhibition of linoleic acid oxidation. The results demonstrate that the ethanolic extract of *O. sipyleum* shows strong antioxidant activity (Table 1). The level of antioxidant properties could be sufficient for this plant to be a natural source of antioxidant substances for use in the food industry as a natural additive.

The results of the phosphomolybdenum assay presented in Table 1 indicate that ethanolic extract from *O. sipyleum* possesses antioxidant capacities. The antioxidant activity of extracts depended on the presence of polyphenols [33] and the powerful antioxidant activity of the ethanolic extract of *O. sipyleum* may be attributed to the presence of polyphenols.

3.3. Metal chelating and Reducing power activity

The metal chelating and reducing abilities of the ethanol extract from *O. sipyleum* were measured in this study and the results shown in Table 1. The results indicate that the ethanol extract demonstrated potential metal chelating and power reducing antioxidant capacity.

Lipid peroxidation can be activated by metal ions starting a chain reaction bringing about the deterioration of food. For this reason, metal chelating capacity is critical as it decreases the volume of catalyzing transition metal in lipid peroxidation [34, 35]. The chelating abilities of the plants contribute directly to their antioxidant properties and can be considered as important mechanisms in the oxidation process. The metal chelating ability of *O. sipyleum* and EDTA were found to be statistically significant ($t=21.03$, $df=7$, $p<0.001$) (Table 1) and the ethanol extract of *O. sipyleum* is capable of chelating Fe^{+2} ions and the chelating agents existing in plant extracts have the ability to reduce the radical formation that can cause damage to living cells [36].

The reducing ability of extracts depends on the presence of polyphenols, which may act as reductones that exert antioxidant action by breaking the free radical chains by donating a hydrogen atom [37]. It has been reported that there is a correlation between total phenolic content and reducing power and metal chelating activity [38, 39].

In the present study, the reducing ability of ethanol extract from *O. sipyleum* was measured and the result of this activity demonstrated that the ethanol extract exhibited a high reduction ability (Table 1). The reducing power and metal chelating activities of *O. sipyleum* may be dependent on total phenolic content.

3.4. Total phenolic, flavonoid and tannin contents

In the present study, the total phenolic, flavonoid and tannin content in the ethanol extract from *O. sipyleum* was determined. The total phenolic, flavonoid and tannin content of ethanol extract were 203.57 ± 4.62 mgGAE/g, 46.98 ± 0.34 mgQE/g and 34.55 ± 0.56 mgCE/g respectively. The phenolic content of the ethanol extract determined in this study were lower than in the methanol extract from *O. sipyleum* as reported by Ozkan et al. [9].

Phenolic compounds (phenolic acid, flavonoid, tannin etc.) in plants possess antioxidant effects and the antioxidative properties of polyphenols based on their various abilities, as in their high reactivity as hydrogen or electron donating agents and, their metal chelating and radical scavenging properties [40].

Although, many reports indicate that besides polyphenols, nonphenolic components present in plants, such as ascorbates, carotenoids, and pigments contribute to the total antioxidative activities [41], phenolic compounds are responsible for a major part of the antioxidant activity of many plants [28]. Establishing the phenolic compounds of plants therefore plays a significant role in the identification of their medicinal properties. In the present study it was observed that there was high phenolic content and high antioxidant activity in the ethanol extract of *O. sipyleum* indicating that the phenolic compounds present in the extracts are largely responsible for antioxidant activity.

3.5. Phenolic composition

Lamiaceae species comprise chemical compounds with biological activities. In this work, phenolic compositions of ethanol extract of *O. sipyleum* were identified using the HPLC method. The phenolic compounds determined in the ethanol extract are listed in Table 2 and the main phenolics were identified as caffeic acid, epicatechin and 2,5 dihydroxybenzoic.

Table 2. Phenolic components of *O. sipyleum*

No	Phenolic component	Retention time (min)	µg/g*
1	2,5 dihydroxybenzoic acid	17.2	4435.68
2	Chlorogenic acid	18.2	75.86
3	3,4 dihydroxybenzoic acid	10.7	72.04
4	4-hydroxybenzoic acid	15.7	147.27
5	Cinnamic acid	71.1	801.74
6	Quercetin	70.4	2591.22
7	Ferulic acid	30.1	19.77
8	*p*-Coumaric acid	26.1	68.90
9	Gallic acid	6.8	138.14
10	Caffeic acid	22.7	16787.16
11	Vanilic acid	19.2	312.51
12	Epicatechin	21.3	4653.17
13	Rutin	45.6	50.48

*based on dry weights

Caffeic acid exhibits anticarcinogenic properties that acts as a carcinogenic inhibitor [42]. The phenolic content that contribute to the antioxidant capacity of the plants [34]. These data indicated that the biological activities of *O. sipyleum* could be attributed to their polyphenol compounds.

3.6. Cytotoxic activity

The brine shrimp cytotoxic bioassay is considered to be useful tool for the preliminary assessment of general toxicity and for estimating the medium lethality concentration LC_{50} [26, 43] and universally as a test for detecting cytotoxic effects. In addition, the brine shrimp cytotoxic bioassay is highly sensitive to a variety of chemical substances [44] and only a small amount of sample is required [45].

Origanum plants from Lamiaceae family are commonly used as herbal teas, flavoring agents and medicinal plants due to their biological and pharmacological properties [8, 9]. *O. sipyleum* is one of the endemic species of the *Origanum* genus and the vegetative parts of this plant have been used as medicinal tea [9]. The lethality of the ethanol extract from *O. sipyleum* was 327.414 µg/mL (LC_{50} < 1000 µg/mL), possessed high cytotoxic activities against brine shrimp and accepted as bioactive due to lower LC_{50}. To date, various studies have been reported that Lamiaceae plants provide a rich source of phytochemical components which are considered to be the basis of various biological activities, including antioxidant, antibacterial, anthelmintic and cytotoxic activity [6, 9, 19, 46, 47]. Accordingly, it could be attributed that the presence of these active components are the basis of the cytotoxic activity. The deadly effect obtained from present study indicates the presence of potent cytotoxic components in this plant and is one that requires further investigation as according to the findings this plant extract possesses a cytotoxic effect.

4. CONCLUSION

The findings in the present work show that in different assays *O. sipyleum* possess antioxidant properties and they also show that the plant possesses rich phenolic, flavonoid and tannin compounds. Furthermore, the ethanol extract show LC_{50} values of less than 1000 µg/mL and this result indicates that ethanol extract of this plant possesses strong cytotoxic activity. According to these findings, this plant could be considered as a source of natural agents in the food industry and could be used as a new cytotoxic agent for pharmacological applications. However, further research would be required before such uses could be proposed with confidence.

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5. REFERENCES

[1] Arouma, O.I. (2010). Free radicals, oxidative stress and antioxidants in human health and diseases. *J. Am. Oil Chem. Soc*., *75*, 199-212.

[2] Lee, J., Koo, N., and Min, D.B. (2004). Reactive oxygen species, aging, and antioxidative nutraceuticals. *Comp. Rev. Food Sci. Food Saf*., *3*, 21–33.

[3] Aktumsek, A., Zengin, G., Guler, G.O., and Cakmak Y.S. (2013). Antioxidant potentials and anticholinesterase activities of methanolic and aqueous extracts of three endemic Centaurea L. species. *Food Chem Toxicol*., *55*, 290-296.

[4] Barlow, S. and Schlatter, J. (2010). Risk assessment of carcinogens in food. *Toxicol. Appl. Pharm*., *243*, 180-190.
[5] Gu, L., Wu, T., and Wang, Z. (2009). TLC bioautography-guided isolation of antioxidants from fruit of *Perilla frutescens* var. acuta. *LWT Food Sci Technol*., *42*, 131-136.
[6] Nickavar, B. and Esbati, N. (2012). Evaluation of the Antioxidant Capacity and Phenolic Content of three *Thymus species. J Acupunct Meridian Stud*., *5*(3), 119-125.
[7] Loziene, K., Venskutonis, P.R., Sipailiene A. and Labokas J. (2007). Radical scavenging and antibacterial properties of the extracts from different *Thymus pulegioides* L. chemotypes. *Food Chem*.,*103*, 546-549.
[8] Nakipoglu, M., Ozturk Urek, R., Ayar Kayali, H., and Tarhan, L. (2007). Antioxidant capacities of endemic *Sideritis sipylea* and *Origanum sipyleum* from Turkey. *Food Chem*., *104*, 630-635.
[9] Ozkan, G., Sagdıç, O., Ekici, L., Ozturk, I., and Özcan, M.M. (2007). Phenolic compounds of *Origanum sipyleum* L. extract, and its antioxidant and antibacterial activities. *J Food Lipid*, *14*, 157-169.
[10] Vazirian, M., Mohammadi, M., Farzaei, M.H.,. Amin, G., and Amanzadeh, Y. (2015) Chemical composition and antioxidant activity of Origanum vulgare subsp. vulgare essential oil from Iran. *Research Journal of Pharmacognosy*, 2(1), 41-46
[11] Coccimiglio, J., Alipour ,M., Jiang, Z.H., Gottardo, C., and Suntres, Z. .(2016). Antioxidant, antibacterial, and cytotoxic activities of the ethanolic *Origanum vulgare* extract and its major constituents, *Oxid Med Cell Longev*. http://dx.doi.org/10.1155/2016/1404505.
[12] Stanojević, L.P.,. Stanojević, J.S., Cvetković, D.J. and Ilić, D.P. (2016). Antioxidant activity of oregano essential oil (Origanum vulgare L.). Biologica Nyssana, *7*(2), 131-139.
[13] Taskin, T., Sadikoglu, N., Birteksoz-Tan, S., and Bitis, L. (2017). *In vitro* screening antioxidant and antimicrobial properties of five commercial *Origanum* species from Turkey. *Indian J. Tradit Knowle*., *16*(4), 568-575.
[14] Karagöz, A., Artun, F.T., Özcan, G., Melikoğlu, G., Anıl, S., Kültür, S., and Sütlüpınar, N. (2015) *In vitro* evaluation of antioxidant activity of some plant methanol extracts. *Biotechnol Biotechnol Equip*., *29*(6), 1184-1189.
[15] Shalaby, E.A. and Shanab S.M.M. (2013) Comparison of DPPH and ABTS assays for determining antioxidant potential of water and methanol extracts of *Spirulina platensis. Indian J Geo Marine Sci*., *42*(5), 556-564.
[16] Meriga, B., Mopuri, R. and Krishna, T.M. (2012). Insectisal antimicrobial and antioxidant activities of bulb extracts of *Allium sativum. Asian Pac J Trop Med*.,. 5(5), 391-395.
[17] Amin, I. and Tan S.H. (2002). Antioxidant activity of selected seaweeds. *Malays J Nut*., *8*, 167-177.
[18] Prieto, P., Pineda M. and Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Anal Biochem*., *269*(2), 337–341.
[19] Kaska, A., Deniz N, Cicek M. and. Mammadov R. (2018). Evaluation of antioxidant properties, phenolic compounds, anthelmintic, and cytotoxic activities of various extracts isolated from Nepeta cadmea: an endemic plant for Turkey. *J Food Sci*., *83*(6), 1552-1559.
[20] Oyaizu, M. (1986). Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition*, *44*(6), 307-316.
[21] Karpagasundari, C. and Kulothungan, S. (2014). Free radical scavenging activity of *Physalis minima* Linn. leaf extract (PMLE). *J Med Plants Stud*., *2*(4), 59-64.
[22] Slinkard, K. and Singleton V.L. (1977). Total phenol analyses: Automation and comparison with manual methods. *Am J Enol Vitic*., *28*, 49–55.

[23] Arvouet-Grand A, Vennat B, Pourrat A, Legret P. (1994). Standardization of a propolis extract and identification of the main constituents. *J Pharm de Belg*., *49*, 462-8.
[24] Broadhurst, R.B., and Jones, W.T. (1978). Analysis of condensed tannins using acidified vanillin. *J Sci Fd Agric*., *29*, 788-794.
[25] Caponio, F., Alloggio, V. and Gomes, T. (1999). Phenolic compounds of virgin olive oil: influence of paste preparation techniques. *Food Chem*., *64*, 203-209.
[26] Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen L.B., Nichols, D.E. and McLaughlin., J.L. (1982). Brine Shrimp: A convenient general bioassay for active plant constituents. *Planta Med*., *45*, 31-34.
[27] Du, G.R., Li, M.J., Ma, F.W., and Liang, D. (2009) Antioxidant capacity and the relationship with polyphenol and Vitamin C in Actinidia fruits. *Food Chem*., *113*, 557-562.
[28] Zengin, G. and Aktumsek, A. (2014). Investigation of antioxidant potentials of solvent extracts from different anatomical parts of *Asphodeline anatolica* E. Tuzlaci an endemic plant to Turkey. *Afr J Tradit Complement Altern Med*., *11*, 481–488.
[29] Kahkonen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J. P., Pihlaja, K., Kujala, T. S. and Heinonen M. (1999). Antioxidant activity of plant extracts containing phenolic compound. *J. Agric. Food Chem*., *47*, 3954-3962.
[30] Maisuthisakul, P., Suttajit M. and Pongsawatmanit R. (2007) Assessment of phenolic content and free radical scavenging capacity of some Thai indigenous plants. *Food Chem*., *100*(4), 1409-1418.
[31] Roby, M.H.H., Sarhan M.A., Selim K.A.H. and. Khalel K.I. (2013). Evaluation of antioxidant activity, total phenols and phenolic compounds in thyme (*Thymus vulgaris L*.), sage (*Salvia officinalis* L.), and marjoram (*Origanum majorana* L.) extracts. *Ind Crops Prod., 43*, 827-831.
[32] Lagouri, V., Guldas, M., and Gurbuz, O. (2011). In vitro Antioxidant/Free Radical Scavenging and Antibacterial Properties of Endemic *Oregano* and *Thyme* Extracts from Greece. *Food Sci. Biotechnol*., *20*(6), 1487-1493
[33] Jayaprakasha, G.K., Selvi, T. and Sakariah, K.K. (2003). Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extracts. *Food Res.Int*., *36* (2), 117-122..
[34] *Duh, P.D., Tu, Y.Y., and Yen, G.C. (1999) Antioxidant activity of water extract of Harug Jyur (Chrysanthemum morifolium Ramat). Lebensmittel Wissenchaft und Technologie. 32(5), 269-277.*
[35] Gordon, MH. (1990) The mechanism of antioxidant action *in vitro*. In B. J. F. Hudson (Ed.), Food antioxidants, 1–18. London/New York: Elsevier.
[36] Al-Dabbas, M.M. (2017). Antioxdant activity of different extracts from the aerial part of Moringa peregrina (Forssk.) Fiori, from Jordan. *Pak J Pharm Sci*., *30*(6), 2151-2157.
[37] Shimada, K., Fujikawa, K., Yahara, K., and Nakamura, T. (1992). Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *J. Agric. Food Chem*., *40*, 945-948.
[38] Zengin, G., Uysal, S., Ceylan, R., and Aktumsek, A. (2015). Phenolic constituent, antioxidative and tyrosinase inhibitory activity of Ornithogalum *narbonense* L. from Turkey: A phtochemical study. *Ind Crop Prod*., *70*, 1-6.
[39] Uysal, S, Zengin, G. and Aktümsek, A. (2016). Antioxidant properties and enzyme inhibitory effects of extracts from *Mandragora autumnalis* and its fatty acid composition. *Marmara Pharm J*., *20*, 144-151.
[40] Rice-Evans, C.A., Miller, N.J., and Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends Plant Sci., 2*, 152-159.

[41] Babbar N., Oberoi H.S., Uppal D.S., and Patil R.T. (2011). Total phenolic content and antioxidant capacity of extracts obtained from six important fruit residues. *Food Res Int*., *44*, 391-396.

[42] Magnani, C, Isaac, V. L. B., Correa, M.A., and Salgado, H.R.N. (2014). Caffeic acid: a review of its potential use in medications and cosmetics. *Anal. Methods*, *6*, 3203-3210.

[43] Krishnaraju, A.V., Rao, T.V.N., Sundararaju, D., Vanisree, M., Tsay, H.S., and Subbaraju, G.V. (2005). Assessment of bioactivity of Indian medicinal plants using Brine Shrimp (*Artemia salina*) lethality assay. *Int. J App. Sci. Eng*., *3*(2), 125-134.

[44] Svoboda, K.P., and Hampson, J.B. (1999). Bioactivity of essential oils of selected temperate aromatic plants: Antibacterial, Antioxidant, Anti-Inflammatory and other related pharmacological activities, Plant Biology Department, SAC Auchincruive, Ayr, Scotland, UK., KA6 5HW.

[45] Hossain, A.M., Ferdous, T., Salehuddin, S.M., and Das, A.K. (2009). *In vitro* cytotoxicity (LC_{50}) of extracts obtained from the seeds of *Zea mays*. *As. J. Food Ag-Ind*., *2*(3), 336-34.

[46] Canadanovic-Brunet, M.J., Dilas, M.S., Cetkovic, S.G., Tumbas, T.V., Mandic, I.A., and Canadanovic M.V. (2006). Antioxidant activities of different *Teucrium montanum* L. extracts. *Int J Food Sci Technol*, *41*, 667-673.

[47] Aksoy-Sagirli, P., Ozsoy, N., Ecevit-Genc, G., and Melikoglu, G. (2015). In vitro antioxidant activity, cyclooxygenase-2, thioredoxin reductase inhibition and DNA protection properties of Teucrium sandrasicum L. *Ind Crops Prod*, *74*, 545-550.