

The screening of *Digitalis ferruginea* L. subsp. *ferruginea* for toxic capacities, phenolic constituents, antioxidant properties, mineral elements and proximate analysis

Arzu KASKA^{1*} , Nahide DENİZ², Mehmet ÇIÇEK³, Ramazan MAMMADOV⁴

Abstract

The present study outlines the antioxidant and toxic activity, total phenol, flavonoid and tannin content of *Digitalis ferruginea*'s extracts in addition to evaluating its proximate parameters and mineral elements. Successive extraction was made using different solvents (ethanol, acetone, water). Total phenol content was determined by the Folin-Ciocalteu's reagent method and flavonoid was determined by the aluminum chloride colorimetric method. The antioxidant activities were investigated using different assays. The phenolics were determined using HPLC. In addition an evaluation was made of the proximate parameters and mineral elements. The radical scavenging capacities were highest in the water extract. The same extract was effective in total antioxidant activities (β -carotene, 83.75%). The acetone extract demonstrated stronger reducing power and phosphomolybdenum antioxidant activity with 0.52 mg/mL and, 107.43 μ g/mg, respectively. The HPLC results determined major phenolics: rutin and ferulic acid. This plant also have rich in polyphenolic content together with toxic activity and possesses nutrients and mineral elements. As well as demonstrating the good antioxidant activity of *D. ferruginea* this study suggest that the plant could be of particular interest from a practical perspective, as it is a significant source of potential natural antioxidants that can be used for the prevention of a range of diseases.

Keywords: *Digitalis ferruginea* L. subsp. *ferruginea*; antioxidant; proximate; toxic.

Practical Application: *D. ferruginea* L. subsp. *ferruginea* may be considered an alternative source of antioxidant and toxic agents for pharmacological applications.

1 Introduction

It is widely acknowledged that reactive oxygen species are closely associated with a range of human diseases, including cancer, inflammation and aging. It is also generally accepted that natural antioxidants could be used as opposed to synthetic compounds to inhibit the growth and development of these reactive species-related disorders in human beings. It is due to their apparent safety and therapeutic value that the natural antioxidants present in plants continue to attract extensive interest. Furthermore, this increased interest in natural antioxidants has in turn resulted in the evaluation of antioxidant presence and levels in many vegetable, fruit, herb, spice and cereal species. As a result, many researchers are looking for natural antioxidants that are potent but safe, in particular those sourced from medicinal plants (Elmastaş et al., 2006; Moein et al., 2008; Rufino et al., 2010; Santos & Gonçalves, 2016).

The genus *Digitalis* belongs to the Plantaginaceae family, which comprises approximately 36 species and is distributed through Europe, the Mediterranean regions and Western Asia (Davis, 1978). There are various species among the genus *Digitalis* that have been shown to have different pharmacological actions, including, antioxidant and antimicrobial activities (Benli et al., 2009; Tusevski et al., 2014; Katanić et al., 2017). In addition, some

members of this genus are used in traditional Turkish medicine as diuretics and tonics (Benli et al., 2009); however, there is still not much known about the antioxidant and toxic activities, phenolic compounds, mineral elements and proximate analysis of *Digitalis* species. *Digitalis ferruginea* L. subsp. *ferruginea* is one of the plant species belonging to the genus *Digitalis*. In previous research, methanol extract from *D. ferruginea* L. subsp. *ferruginea* has been investigated for phenolic compounds, antioxidant, antimicrobial and enzyme inhibitory activities (Katanić et al., 2017). Nevertheless, it is well known note that even within a single method a small difference in solvent polarity may provide different responses, due to the diversity of the chemical nature of these compounds and their often unique distribution within the plant matrix (Antolovich et al., 2000; Turkmen et al., 2006; Sultana et al., 2009). For this reason, the antioxidant efficacy of the resulting extracts is strongly affected, as mentioned above, by a polarity of the solvent and the chemical nature of the isolated compounds (Sultana et al., 2009; Shabir et al., 2011). This varies, depending on the type of solvent used and in addition polarity may modify the single electron transfer and the hydrogen atom transfer, both of which are key with regard to the measurement of antioxidant capacity (Pérez-Jiménez & Saura-Calixto, 2006). Thus, a significant contribution could be made to medicinal plant

Received: 24 Feb., 2020

Accepted: 05 May, 2020

¹Department of Science and Mathematics, Faculty of Education, Pamukkale University, Denizli, Turkey

²Department of Biology, Faculty of Arts and Science, Pamukkale University, Denizli, Turkey

³Department of Molecular Biology and Genetics, Faculty of Arts and Science, Pamukkale University, Denizli, Turkey

⁴Department of Molecular Biology and Genetics, Faculty of Science, Sıtkı Koçman University, Muğla, Turkey

*Corresponding author: akaska@pau.edu.tr, akaska@pau.edu.tr

studies through the discrete analysis of plant extracts acquired from a variety of solvents (Canadanovic-Brunet et al., 2006; Stankovic et al., 2011). In the current study, we therefore investigated antioxidant properties using different methods, toxic effects and total bioactive compounds of the ethanol, acetone and water extracts of *D. ferruginea* L. subsp. *ferruginea* and to evaluate this plant's mineral elements and proximate analysis. In addition we analyzed the phenolic compound of ethanolic extract of *D. ferruginea* L. subsp. *ferruginea*, for a better characterization and exploitation of this natural product.

2 Materials and methods

2.1 Plant material and preparation of the extracts

The aerial parts of *Digitalis ferruginea* L. subsp. *ferruginea* were collected at the flowering stage, from Denizli, Turkey, in August 2018. The plant material was identified and stored with voucher specimens (*D. ferruginea* L. subsp. *ferruginea*; Herbarium No: 2018-5-2) at the private herbarium of Dr. M. Çiçek. To obtain the extracts, the sample (10 g) was added to 100 mL of solvents (ethanol, acetone and water) and shaken at 50 °C for 6 h in a temperature controlled shaker. The extracts were filtered twice and solvents from the samples were removed using a rotary evaporator. The samples were dried in a lyophilizer and kept at -20 °C until tested. Each experiment was conducted in triplicate.

2.2 Antioxidant activity

The Phosphomolybdenum and the β -carotene/linoleic acid bleaching methods were used to ascertain the antioxidant properties. The antioxidant capacity with phosphomolybdenum method was reported as ascorbic acid equivalents. β -carotene/linoleic acid bleaching assay was used to BHT and TROLOX as standards. These methods were described in our published paper (Kaska et al., 2018). The radical scavenging (DPPH and ABTS) and metal chelating activities were evaluated by the method described by Kaska et al., 2018, and these assays were used to EDTA, BHT and TROLOX as standards. The ferric ion reducing antioxidant power procedure followed was those given by Kaska & Mammadov (2019a), and results were expressed as mg of ascorbic acid equivalents (AA) per milliliter of extract.

2.3 Quantification of phenolic compounds by HPLC

The phenolic compound were evaluated using Reversed-phase high performance liquid chromatography (RP-HPLC, Shimadzu Scientific Instruments). The phenolic composition of the ethanolic extract of *D. ferruginea* L. subsp. *ferruginea* was determined using previously described method Caponio et al. (1999). The details of this method were given in Kaska & Mammadov (2019a). 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. The quantitative analysis were made by comparing the standards. The results were expressed as $\mu\text{g/g}$ of each compound from the total phenolic compounds.

2.4 Total bioactive compounds

Folin-Ciocalteu and aluminum chloride colorimetric assays were used to determine the total phenolic and flavonoid contents, respectively (Slinkard & Singleton, 1977; Arvouet-Grand et al., 1994). To evaluate the tannin content was performed according to Broadhurst & Jones (1978) using the vanillin-HCl method. The results were expressed as the equivalents of Gallic acid (mgGAE g^{-1}), Quercetin (mgQEs g^{-1}) and Catechin (mgCEs g^{-1}) for phenolic, flavonoid and tannin content respectively. The procedure for determining the bioactive compounds was conducted according to Kaska et al. (2018).

2.5 Proximate composition

The *D. ferruginea* L. subsp. *ferruginea* was analyzed to investigate the proximate parameters (proteins, fat, carbohydrates, ash and energy) according to the Association of Official Analytical Chemists (1995) protocols. The sample preparation and procedure for determining the proximate parameters were followed according to Kaska & Mammadov (2019b).

2.6 Mineral elements

The mineral elements (potassium, magnesium, Phosphorus, Iron and Copper) were evaluated using ICP-OES method (optical emission spectrometer with the inductively coupled plasma). The details of this method were given in Kaska et al. (2019). The samples of the studied herbs were mineralized in nitric acid 65% HNO_3 . The standard solution were prepared by diluting (P, Mg, Fe, K, Cu) (Merc) standard solutions at the concentrations of 1000 ppm (mg/L). For the preparation of standard solution deionized water was used. In order to determine the calibration curve, standard solutions at the range of concentrations from (0.005-1 ppm) were used. The study used the analytical lines of the highest intensity. An analysis of samples for the presence of elements was carried out using an optical emission spectrometer with the inductively coupled plasma.

2.7 Brine shrimp lethality assay

The brine shrimp lethality bioassay was used to investigate the toxicity of *D. ferruginea* L. subsp. *ferruginea* (Meyer et al., 1982). The details of determining the toxicity were given in our previous study (Kaska & Mammadov, 2019a). The EPA Probit Analysis Program was used for data analysis.

2.8 Statistical analysis

The experimental results were analyzed using the MINITAB Statistical Package program and the results expressed as mean \pm SE (Standard Error). The differentiations between the extracted groups were tested using ANOVA (Analysis of Variance) and then Tukey was conducted ($p < 0.05$).

3 Results and discussion

The antioxidant capacity of plants is derived from the synergistic action of a wide variety of antioxidants and one single assay cannot fully describe the antioxidant activity. To determine the antioxidant capacity of plants *in vitro*, it is normally required to integrate

several methods (Pérez-Jiménez & Saura-Calixto, 2006; Santos & Gonçalves, 2016). In the present study, various assays were conducted to analyze antioxidant activities of the extracts.

3.1 Antioxidant capacity

The phosphomolybdenum assay is contingent on the extract's reduction of Mo (VI) to Mo (V) and the development of green phosphate/Mo(V) complex. The reducing capacities are usually associated with the occurrence of reductones (Prieto et al., 1999). The findings for the antioxidant evaluation given in Table 1 indicate that ethanol, acetone and water extracts from *D. ferruginea L. subsp. ferruginea* possess antioxidant capacities.

In this study, according to the results of the inhibition of linoleic acid oxidation for ethanol, acetone and water extracts, the ethanol extraction of aerial parts displays lower antioxidant values ($75.73 \pm 1.54\%$), than water extraction ($83.75 \pm 0.88\%$) (Figure 1).

Ethanol and water extracts showed significant differences ($p < 0.001$) when compared with the antioxidant activity value of BHT and TROLOX, which were $93.71 \pm 0.23\%$ and $94.81 \pm 1.15\%$, respectively. Among the extracts investigated, acetone extract exhibited the lowest antioxidant activity ($74.49 \pm 0.96\%$) and its activity was to be significantly different from the BHT and TROLOX antioxidant activities ($F_{4,35} = 85.10$ $p < 0.001$). The extracts studied in this study were found to effectively inhibit linoleic acid oxidation. These findings demonstrate that they possess strong antioxidant capacities. When the ethanol, water and acetone extracts of *D. ferruginea L. subsp. ferruginea* were compared with the methanol extract from *D. ferruginea L. subsp. ferruginea* (Katanić et al., 2017) of which the total antioxidant activity was found to be 78.59%, the antioxidant activity of the ethanol and acetone extracts were lower while the water extract's antioxidant activity was higher than those of the methanol extract of this species.

Radical scavenging activities are essential because of the detrimental role of free radicals in foodstuffs and biological systems. ABTS and/or DPPH scavenging assays are common spectrophotometric methods for analyzing the radical scavenging activities (Gülçin et al., 2010; Olszowy & Dawidowicz, 2018). The DPPH assay offers an effective, quick and simple way to assess potential antioxidants. This method is an antioxidant assay that uses an electron transfer and in combination with ethanol produces a violet solution. Likewise, stable at room temperature, this free radical is reduced in the presence of an antioxidant molecule, resulting in a yellow solution (Vlase et al., 2014).

In the present study, the radical scavenging activity of different extracts of *D. ferruginea L. subsp. ferruginea* is expressed in terms of IC_{50} (mg/mL) values (Table 1). A reduction in the IC_{50} value signifies a higher level of antioxidant activity. In tandem with the analysis of antioxidant activities of the plant extracts, the values for two standard compounds were attained and equated with those of the antioxidant activity. The DPPH free radical scavenging activity of the water, ethanol and acetone extracts were significantly different from each other and IC_{50} values of all extracts were found to be different from BHT and TROLOX IC_{50} values ($F_{4,35} = 1831.22$ $p < 0.001$). Katanić et al., (2017) used DPPH for the determination of radical scavenging activity in methanol extracts from *D. ferruginea L. subsp. ferruginea*. In the current investigation, The DPPH assay was used to examine scavenging activity in ethanol, water and acetone extracts from *D. ferruginea L. subsp. ferruginea* and found that the extracts of *D. ferruginea L. subsp. ferruginea* have radical scavenging activity.

The ABTS assay, used for analyzing hydroxybenzoic and hydroxycinnamic acids, which, were due to their hydrogen and electron donating capacity, together with their ability to stabilize the ensuing phenoxyl radical within the structure, capitalizes on the strong likelihood of their acting as radical scavengers (Silva et al., 2001; Biskup et al., 2013). In the ABTS scavenging assay, the IC_{50} values of standards and different extracts are given in Table 1. The acetone, ethanol and water extracts tested in our study demonstrated a scavenging capacity. These samples were significantly different from each other and the IC_{50} values of the all extracts were found to be statistically different from the BHT and TROLOX IC_{50} values ($F_{4,35} = 543.72$ $p < 0.001$).

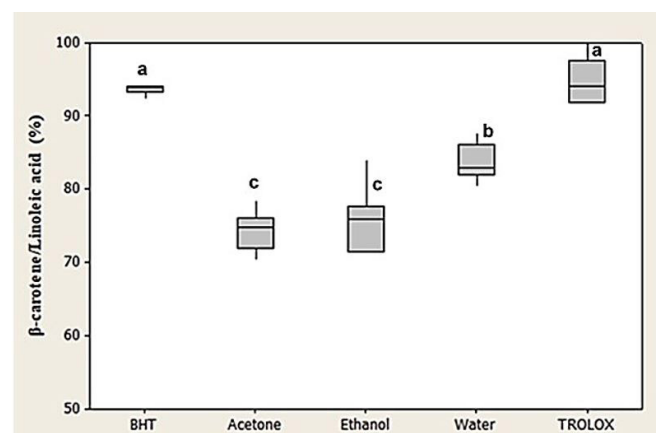


Figure 1. The β -carotene/linoleic acid activity of *D. ferruginea L. subsp. ferruginea*.

Table 1. Antioxidant properties of *D. ferruginea L. subsp. ferruginea*.

Sample	DPPH (IC_{50} , mg/mL)	ABTS (IC_{50} , mg/mL)	Phosphomolybdenum (μ g/mg)	Power reducing (mg/mL)
Ethanol	0.610 ± 0.01 b	0.473 ± 0.02 a	73.32 ± 7.08 b	0.51 ± 0.04 b
Acetone	1.291 ± 0.02 a	0.472 ± 0.01 a	107.43 ± 4.41 a	0.52 ± 0.05 b
Water	0.438 ± 0.02 c	0.415 ± 0.01 b	94.81 ± 4.43 a	0.44 ± 0.03 b
TROLOX	0.021 ± 0 d	0.026 ± 0 c	nt	nt
BHT	0.032 ± 0 d	0.027 ± 0 c	nt	1.15 ± 0.02 a
EDTA	nt	nt	nt	nt

The statistical differences were given as different letters ($p < 0.05$). nt: not tested; DPPH: 2,2-Diphenyl-1-picrylhydrazyl radical; ABTS: 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid.

According to the findings, the water extract showed remarkable radical scavenging antioxidant activity with 0.415 mg/mL.

Free radicals are electrically charged, unstable molecules that are formed as a result of the metabolic processes of cells or by external factors such as air and it is possible for them to attack the healthy cells of the body, resulting in them losing their structure and function, and the cell damage caused by free radicals appears to be a major contribution to degenerative diseases of aging, such as cardiovascular disease, heart disease, diabetes, and liver diseases (Mohan et al., 2012; Phaniendra et al., 2015). Likewise, free radicals involved in the lipid peroxidation process are thought to play a role in various diseases, such as cancer (Dorman et al., 2003). For this reason, researchers have nowadays become increasingly interested in natural resources that could produce active components to prevent free radicals negatively impacting on cells (Tepe et al., 2007). The acetone, ethanol and water extracts of *D. ferruginea* L. subsp. *ferruginea* tested in the present study each showed a radical scavenging capacity.

In our study, *D. ferruginea* L. subsp. *ferruginea* extracts were analyzed to determine their Fe^{2+} chelating activity. Then the results were contrasted with the chelating activity of the synthetic metal chelator EDTA ($88.27 \pm 1.18\%$). The water extract chelated more iron ($46.13 \pm 0.87\%$) than ethanol and acetone extracts with chelating values of 27.85 ± 1.86 and $38.47 \pm 1.17\%$, respectively (Figure 2).

The chelating capacity of metal ion is noteworthy, as in lipid peroxidation it can contribute to the reduction of the concentration of the catalyzing transition metal. Chelating agents, which can form bonds with a metal, are moreover as effective as secondary antioxidants, as they can reduce the redox potential and thus stabilize the oxidized form of the metal ion (Elmastaş et al., 2006; Al-Dabbas, 2017). In the current study, *D. ferruginea* L. subsp. *ferruginea* exhibited a clear propensity for iron binding. This indicates their capacity as a peroxidation protector, which in turn correlates with the iron binding capacity.

The reducing power assay method uses the principle that a substance with reduction potential, will react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}). This then reacts with ferric chloride, forming a ferric-ferrous complex,

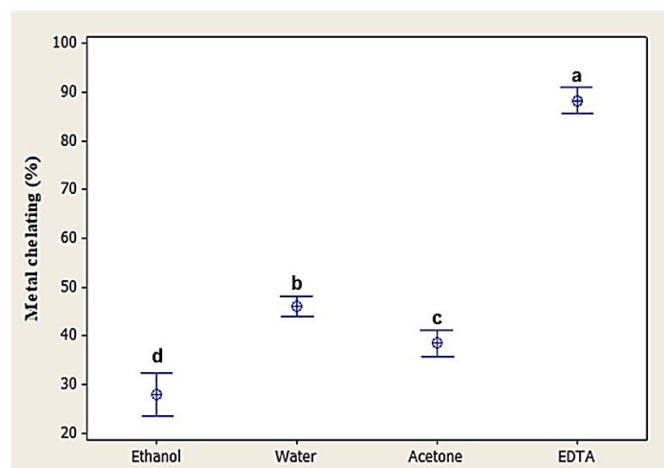


Figure 2. The Metal chelating activity of *D. ferruginea* L. subsp. *ferruginea*.

which has an absorption maximum at 700 nm. In addition, the reducing power assay is a convenient and rapid screening method for measuring the antioxidant potential (Talukder et al., 2013). In the present study, we investigated ethanol, water and acetone extracts from *D. ferruginea* L. subsp. *ferruginea* for reducing capacity and the acetone extract demonstrated stronger reducing power activity with 0.52 mg/mL followed by ethanol and water extracts with 0.51 and 0.44 mg/mL, respectively. The reducing capability of a compound generally depends on the presence of reductants that have been exhibiting antioxidative potential by breaking the free radical chain and donating a hydrogen atom (Duh et al., 1999; Babu et al., 2013). The presence of reductants in *D. ferruginea* L. subsp. *ferruginea* extracts causes the reduction of the Fe^{3+} /ferricyanide complex to the ferrous form.

3.2 Total phenolic, flavonoid, tannin content and phenolic composition

Phenolic compounds are found in plants, and they have been reported to have various biological properties, including antioxidant capacity. For this reason, the richness of the phenolic compounds in plants indicate their pharmacological properties; and analyses of these phenolic compounds are crucial for understanding their medicinal value. Besides this, the food industry is showing increasing interest in plants that are rich in phenolics. This is because they delay the oxidative degradation of lipids and in so doing improve the nutritional value and quality of food (Marimuthu et al., 2008; Tungmunnithum et al., 2018). In brief, the identification and measurement of plants' phenolic compounds are nowadays considered to be effective mechanisms for ascertaining the importance of plants for human health (Amarowicz et al., 2010). The content of the bioactive compound in the different *D. ferruginea* L. subsp. *ferruginea* extracts are existed in Table 2.

The experimental results showed that the highest contents of total phenolic was in the ethanolic extract of *D. ferruginea* L. subsp. *ferruginea* was highest. The phenolic content for the water, ethanol and acetone extracts analyzed in current study were higher than that determined by Katanić et al. (2017) (methanol extract of *D. ferruginea* L. subsp. *ferruginea*). The total flavonoid content in the extracts ranged from 10.45 to 51.73 mgQEs/g and there were statistical differences ($F_{2,21}=4527.11$ $p < 0.001$) in the total flavonoid contents of the various extracts of *D. ferruginea* L. subsp. *ferruginea*. In this study, total phenolic and flavonoid content of the extracts varied in accordance with the solvent. Similar to these results, the studies of Khazai et al. (2011) and Kaska et al. (2019) found that the total phenolic and flavonoid content differed according to the solvents used. As Table 2 shows, tannin content was highest in the acetone extract (94.49 mgCEs/g), and lowest in the water extract (29.99 mgCEs/g). According to the findings in current study, the bioactive compounds were found to be considerable in each of the extracts we investigated.

The results for phenolic compositions of the ethanolic extract of *D. ferruginea* L. subsp. *ferruginea* by HPLC analysis are presented in Table 3, and major phenolics were determined: rutin, ferulic, quercetin and gallic acid.

Rutin is a common dietary flavonoid that has been shown to have powerful antioxidant capacity (Guo et al., 2007; Yang et al., 2008). Quercetin, a plant-derived flavonoid, has been used in traditional medicine to prevent or treat a variety of diseases, such as cancer (Neuhaus, 2004; Murakami et al., 2008), cardiovascular and nervous disorders (Shankar et al., 2007; Labinsky et al., 2006). Ferulic acid belongs to the phenolic acid group commonly found in plant tissues (Mattila & Kumpulainen, 2002) and it possess many physiological functions, such as anti-inflammatory, antithrombotic, antidiabetic effects and it also reduces nerve cell damage. It has also been widely used in the pharmaceutical, food, and cosmetics industry (Tee-ngam et al., 2013; Cota-Arriola et al., 2017; Zduńska et al., 2018). Gallic acid is a naturally occurring polyphenol antioxidant that has recently been shown to have potentially healthy effects (Lu et al., 2006). According to these studies, phenolic compounds isolated from the plant possess a wide range of bioactivities and for this reason, phenolic compounds may be used as an important indicator of the biological activity of plants.

3.3 Proximate composition

The proximate composition of a plants provides valuable information regarding its medicinal and nutritional quality. Proteins and carbohydrates are important nutrients in plants that should be assessed. Fat provides a very good sources of energy and aids in the transportation of fat soluble vitamins, as well as insulating and protecting the internal tissues (Hussain et al., 2013; Ghani et al., 2016). The results of proximate composition of *D. ferruginea* L. subsp. *ferruginea* are presented in Table 4 and the proximate composition of the studied plant in our study is in agreement with previously data reported in the literature. Proximate analysis determinants by Ghani et al., 2016 and Bukhsh et al., 2007, have revealed that the *Plantago ovata* from Plantaginaceae family contained carbohydrate (30.33, 15.9%), and protein (7.12, 21.87%) content.

In addition, our results are in accordance with the results of previous studies in which the ash content ranged from 2.67% to 17.22% in certain plants belonging to Plantaginaceae family (Guil-Guerrero, 2010; Ghani et al., 2016; Ogbiko et al., 2017). The search for pharmacologically active chemicals from plant sources is continuing and today many compounds have been isolated and introduced into clinical medicine (Bukhsh et al., 2007). The present study was performed to further increase knowledge about the proximate composition of Plantaginaceae and its focus was the investigation of proximate composition *D. ferruginea* L. subsp. *ferruginea*.

3.4 Mineral elements

The ICP-OES method was used for the determining the content of P, Mg, K, Fe and Cu in the tested plant. The findings are presented in Table 5.

Mineral elements play important roles in biological, metabolic and enzymatic reactions of living organisms (Mishra et al., 2012; Ghani et al., 2016). Copper and Magnesium are essential elements for living organisms in terms of their presence in important enzymes (Velasco-Reynold et al., 2008; Tomescu et al., 2015). Iron (Fe) is a important component of heme proteins, hemoglobin, and myoglobin (Fraga, 2005). Phosphorus is essential component of bone mineral. Deficiency of phosphorus- calcium balance result in osteoporosis, rickets and tooth decay (Asaolu et al., 2012). Plant's minerals are great importance to understand pharmaceutical

Table 3. Phenolic components in the ethanolic extract of *D. ferruginea* L. subsp. *ferruginea*.

No	Phenolic component	Approximate Rt (min)	µg/g*
1	Gallic acid	6.8	213.16
2	2,5 dihydroxybenzoic acid	17.2	17.67
3	Chlorogenic acid	18.2	1.35
4	3,4 dihydroxybenzoic acid	10.7	50.39
5	4-hydroxybenzoic acid	15.7	6.44
6	Cinnamic acid	71.1	207.27
7	Quercetin	70.4	213.65
8	<i>p</i> -Coumaric acid	26.1	168
9	Ferulic acid	30.1	929.84
10	Caffeic acid	22.7	102.09
11	Vanilic acid	19.2	5.24
12	Epicatechin	21.3	1.68
13	Rutin	45.6	966.05

*based on dry weights. Rt: Retention time.

Table 4. Proximate analysis of *D. ferruginea* L. subsp. *ferruginea*.

Constituents	Aerial parts
Ash (g/100 g dw)	11.14 ± 0.2
Carbohydrate (g/100 g dw)	18.26 ± 0.33
Proteins (g/100 g dw)	4.02 ± 0.1
Fat (g/100 g dw)	0.2 ± 0.02
Energy (kcal/100 g dw)	90.93 ± 1.59

dw: dry weight.

Table 5. Mineral analysis of *D. ferruginea* L. subsp. *ferruginea*.

Mineral content	Aerial parts
Phosphorus (P) (g/kg)	2.37 ± 5.20
Magnesium (Mg) (g/kg)	2.8 ± 0.37
Potassium (K) (g/kg)	16.18 ± 1.42
Iron (Fe) (g/kg)	0.24 ± 10.09
Copper (Cu) (g/kg)	0.1 ± 11.03

Data were given as the mean of the three measurements (n=3) ± standard error.

Table 2. Total bioactive compound of *D. ferruginea* L. subsp. *ferruginea* extracts.

Sample	Total phenolic content (mgGAEs/g)	Total flavonoid content (mgQEs/g)	Total tannin content (mgCEs/g)
Acetone	59.09 ± 1.07 ab	51.73 ± 0.44 a	94.49 ± 4.35 a
Water	56.93 ± 1.34 b	10.45 ± 0.28 c	29.99 ± 1.51 b
Ethanol	61.48 ± 0.79 a	23.48 ± 0.17 b	32.99 ± 0.95 b

The statistical differences were given as different letters (p < 0.05).

actions of plants, therefore, it is important to analyze the mineral contents of plants before recommending them for medicinal uses. According to the findings of present study, *D. ferruginea* L. subsp. *ferruginea* could be a good source of minerals.

3.5 Brine shrimp lethality assay

The brine shrimp lethality assay is a straightforward, high output toxicity test for bioactive chemicals. It is determined by the killing ability of the test compounds of *Artemia salina*, the brine shrimp. This very basic form of zoological organism is widely used in these investigations because of its commercial availability (Nguta et al., 2012; Ahmed et al., 2017). This assay is considered to be a useful tool for the preliminary assessment of general toxicity and for estimating the medium lethality concentration LC_{50} (Meyer et al., 1982). In this study, an experiment for toxic activity was carried out on the ethanol, acetone and water extracts of *D. ferruginea* L. subsp. *ferruginea* collected from Turkey and the toxicity was reported in terms of 50% lethal concentration (LC_{50}). According to our results, the lethality of the ethanol, acetone and water extracts from *D. ferruginea* L. subsp. *ferruginea* were 407.791, 266.954 and 137.069 $\mu\text{g/mL}$, respectively. The LC_{50} values of extracts were found to be lower than 1000 $\mu\text{g/mL}$, and considered significantly active. This significant lethality of the extracts could be the source of potentially toxic components in these species but further investigation is required to find and isolate the chemical compounds that contain these toxic properties. This will in turn enhance our understanding of the plant, its potential and its medicinal uses.

4 Conclusion

Nowadays, demand for the scientific evaluations of the antioxidant properties of plant extracts is intensifying. This rise is due to their being for the most part harmless sources from which to obtain natural antioxidants and in recent years, antioxidant products made from natural sources have been under the spotlight. The results presented here indicate that extracts of *D. ferruginea* L. subsp. *ferruginea* have promising biological effects. The tested extracts have shown good antioxidant and toxic activity. They are also rich in phenolic, flavonoid and tannin content. In addition, this plant possess varied polyphenolic compounds with utility properties as well as mineral elements and proximate composition. Our results also highlight the biological potential of this plant and in addition we believe that they will encourage further studies to be carried out on the isolation and characterization of these bioactive compounds to determine the mechanism of action. Finally, the outcomes of this study could provide significant information regarding *D. ferruginea*'s potential use in the pharmaceutical industry.

Acknowledgements

We would like to thank lab members of the Secondary Metabolites Lab., Pamukkale University, Denizli-Turkey.

References

- Ahmed, M., Fatima, H., Qasim, M., Gul, B., & Ihsan-Ul-Haq, (2017). Polarity directed optimization of phytochemical and in vitro biological potential of an indigenous folklore: *Quercus dilatata* Lindl. ex Royle. *BMC Complementary and Alternative Medicine*, 17(1), 386. <http://dx.doi.org/10.1186/s12906-017-1894-x>. PMID:28774308.
- Al-Dabbas, M. M. (2017). Antioxidant activity of different extracts from the aerial part of *Moringa peregrina* (Forssk.) Fiori, from Jordan. *Pakistan Journal of Pharmaceutical Sciences*, 30(6), 2151-2157. PMID:29175784.
- Amarowicz, R., Estrella, I., Hernandez, T., Robredo, S., Troszynska, A., Kosinska, A., & Pegg, R. B. (2010). Free radical scavenging capacity, antioxidant activity, and phenolic composition of green lentil (*Lens culinaris*). *Food Chemistry*, 121(3), 705-711. <http://dx.doi.org/10.1016/j.foodchem.2010.01.009>.
- Antolovich, M., Prenzler, P. D., Robards, K., & Ryan, D. (2000). Sample preparation in the determination of phenolic compounds in fruits. *Analyst*, 125(5), 989-1009. <http://dx.doi.org/10.1039/b000080i>.
- Arvouet-Grand, A., Vennat, B., Pourrat, A., & Legret, P. (1994). Standardization of a propolis extract and identification of the main constituents. *Journal de Pharmacie de Belgique*, 49(6), 462-468. PMID:7884635.
- Asaolu, S. S., Adefemi, O. S., Oyakilome, I. G., Ajibulu, K. E., & Asaolu, M. F. (2012). Proximate and mineral composition of Nigerian leafy vegetables. *Journal of Food Research*, 1(3), 214-218. <http://dx.doi.org/10.5539/jfr.v1n3p214>.
- Association of Official Analytical Chemists – AOAC. (1995). *Official methods of analysis* (16th ed.). Gaithersburg: AOAC .
- Babu, D., Gurumurthy, P., Borra, S. K., & Cherian, K. M. (2013). Antioxidant and free radical scavenging activity of triphala determined by using different in vitro models. *Journal of Medicinal Plants Research*, 7(39), 2898-2905. <http://dx.doi.org/10.5897/JMPR2013.5124>.
- Benli, M., Yiğit, N., Geven, F., Güney, K., & Bingöl, U. (2009). Antimicrobial activity of endemic *Digitalis lamarckii* Ivan from Turkey. *Indian Journal of Experimental Biology*, 47(3), 218-221. PMID:19405389.
- Biskup, I., Golonka, I., Gamian, A., & Sroka, Z. (2013). Antioxidant activity of selected phenols estimated by ABTS and FRAP methods. *Postępy Higieny i Medycyny Doswiadczalnej*, 67, 958-963. <http://dx.doi.org/10.5604/17322693.1066062>. PMID:24088539.
- Broadhurst, R. B., & Jones, W. T. (1978). Analysis of condensed tannins using acidified vanillin. *Journal of the Science of Food and Agriculture*, 29(9), 788-794. <http://dx.doi.org/10.1002/jsfa.2740290908>.
- Bukhsh, E., Malik, S. A., & Ahmad, S. S. (2007). Estimation of nutritional value and trace elements content of *Carthamus oxyacantha*, *Eruca sativa* and *Plantago ovata*. *Pakistan Journal of Botany*, 39, 1181-1187.
- Canadanovic-Brunet, J. M., Djilas, S. M., Cetkovic, G. S., Tumbas, V. T., Mandic, A. I., & Canadanovic, V. M. (2006). Antioxidant activities of different *Teucrium montanum* L. extracts. *International Journal of Food Science & Technology*, 41(6), 667-673. <http://dx.doi.org/10.1111/j.1365-2621.2006.01133.x>.
- Caponio, F., Alloggio, V., & Gomes, T. (1999). Phenolic compounds of virgin olive oil: influence of paste preparation techniques. *Food Chemistry*, 64(2), 203-209. [http://dx.doi.org/10.1016/S0308-8146\(98\)00146-0](http://dx.doi.org/10.1016/S0308-8146(98)00146-0).
- Cota-Arriola, O., Plascencia-Jatomea, M., Lizardi-Mendoza, J., Robles-Sánchez, R. M., Ezquerro-Brauer, J. M., Ruiz-García, J., Vega-Acosta, J. R., & Cortez-Rocha, M. O. (2017). Preparation of chitosan matrices with ferulic acid: physicochemical characterization and relationship on the growth of *Aspergillus parasiticus*. *CYTA: Journal of Food*, 15, 65-73.
- Davis, P. H. (1978). *Flora of Turkey and the East Aegean Islands*. Edinburgh: Edinburgh University Press.
- Dorman, H. J. D., Peltoketo, A., Hiltunen, R., & Tikkanen, M. J. (2003). Characterization of the antioxidant properties of de-odourised aqueous extracts from selected Lamiaceae herbs. *Food Chemistry*, 83(2), 255-262. [http://dx.doi.org/10.1016/S0308-8146\(03\)00088-8](http://dx.doi.org/10.1016/S0308-8146(03)00088-8).

- Duh, P. D., Tu, Y. Y., & Yen, G. C. (1999). Antioxidant activity of water extract of Harnng Jyur (*Chrysanthemum moifolium* Ramat). *Lebensmittel-Wissenschaft + Technologie*, 32(5), 269-277. <http://dx.doi.org/10.1006/fstl.1999.0548>.
- Elmastaş, M., Gülçin, İ., Beydemir, Ş., İrfan Küfrevioğlu, Ö., & Aboul-Enein, H. Y. (2006). A study on the in vitro antioxidant activity of Juniper (*Juniperus communis* L.) fruit extracts. *Analytical Letters*, 39(1), 47-62. <http://dx.doi.org/10.1080/00032710500423385>.
- Fraga, C. G. (2005). Relevance, essentiality and toxicity of trace elements in human health. *Molecular Aspects of Medicine*, 26(4-5), 235-244. <http://dx.doi.org/10.1016/j.mam.2005.07.013>. PMID:16125765.
- Ghani, A., Ameen, O., Ikram, M., Hussain, M., Ahmad, I., Khan, A., Hameed, T., Iftikhar, M., Imran, M., Farooq, M., Fatima, H., Muhammad, N., Shahzad, K., & Arshad, M. (2016). Proximate and elemental profile of *Plantago ovata*. *International Researchers*, 5, 1-9.
- Guil-Guerrero, J. L. (2010). Nutritional composition of *Plantago* species (*P. major* L., *P. lanceolata* L., and *P. media* L.). *Ecology of Food and Nutrition*, 40(5), 481-495. <http://dx.doi.org/10.1080/03670244.2010.9991663>.
- Gülçin, I., Huyut, Z., Elmastas, M., & Aboul-Enein, H. Y. (2010). Radical scavenging and antioxidant activity of tannic acid. *Arabian Journal of Chemistry*, 3(1), 43-53. <http://dx.doi.org/10.1016/j.arabjc.2009.12.008>.
- Guo, R., Wei, P., & Liu, W. (2007). Combined antioxidants effects of rutin and vitamin C in Triton X-100 micelles. *Journal of Pharmaceutical and Biomedical Analysis*, 43(4), 1580-1586. <http://dx.doi.org/10.1016/j.jpba.2006.11.029>. PMID:17196356.
- Hussain, J., Rehman, N. U., Al-Harrasi, A., Ali, L., Khan, A. L., & Albroumi, M. A. (2013). Essential oil composition and nutrient analysis of selected medicinal plants in Sultane of Oman. *Asian Pacific Journal of Tropical Disease*, 3(6), 421-428. [http://dx.doi.org/10.1016/S2222-1808\(13\)60095-X](http://dx.doi.org/10.1016/S2222-1808(13)60095-X).
- Kaska, A., & Mammadov, R. (2019a). Antioxidant properties, proximate analysis, phenolic compounds, anthelmintic and cytotoxic screening of *Teucrium sandrasicum*, an endemic plant for Turkey. *Italian Journal of Food Science*, 31, 332-346.
- Kaska, A., & Mammadov, R. (2019b). Antioxidant properties, proximate content and cytotoxic activity of *Echinophora tournefortii* Jaub. & Spach. *Food Science and Technology*, 39(4), 875-880. <http://dx.doi.org/10.1590/fst.09118>.
- Kaska, A., Çiçek, M., & Mammadov, R. (2019). Biological activities, phenolic constituents and mineral element analysis of two endemic medicinal plants from Turkey: *Nepeta italica* subsp. *cadmea* and *Teucrium sandrasicum*. *South African Journal of Botany*, 124, 63-70. <http://dx.doi.org/10.1016/j.sajb.2019.04.037>.
- Kaska, A., Deniz, N., Cicek, M., & 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. *Journal of Food Science*, 83(6), 1552-1559. <http://dx.doi.org/10.1111/1750-3841.14167>. PMID:29746001.
- Katanić, J., Ceylan, R., Matić, S., Boroja, T., Zengin, G., Aktumsek, A., Mihailović, V., & Stanić, S. (2017). Novel perspectives on two *Digitalis* species: phenolic profile, bioactivity, enzyme inhibition, and toxicological evaluation. *South African Journal of Botany*, 109, 50-57. <http://dx.doi.org/10.1016/j.sajb.2016.12.004>.
- Khazai, V., Piri, K. H., Nazeri, S., Karamian, R., & Zamani, N. (2011). Free radical scavenging activity and phenolic and flavonoid contents of *Echinophora platyloba* DC. *Asian Journal of Medical and Pharmaceutical Researches*, 1, 9-11.
- Labinskyy, N., Csiszar, A., Veress, G., Stef, G., Pacher, P., Oroszi, G., Wu, J., & Ungvari, Z. (2006). Vascular dysfunction in aging: potential effects of resveratrol, an anti-inflammatory phytoestrogen. *Current Medicinal Chemistry*, 13(9), 989-996. <http://dx.doi.org/10.2174/092986706776360987>. PMID:16611080.
- Lu, Z., Nie, G., Belton, P. S., Tang, H., & Zhao, B. (2006). Structure-activity relationship analysis of antioxidant ability and neuroprotective effect of gallic acid derivatives. *Neurochemistry International*, 48(4), 263-274. <http://dx.doi.org/10.1016/j.neuint.2005.10.010>. PMID:16343693.
- Marimuthu, P., Wu, C. L., Chang, H. T., & Chang, S. T. (2008). Antioxidant activity of the ethanolic extract from the bark of *Chamaecyparis obtusa* var. *formonasa*. *Journal of the Science of Food and Agriculture*, 88(8), 1400-1405. <http://dx.doi.org/10.1002/jsfa.3231>.
- Mattila, P., & Kumpulainen, J. (2002). Determination of free and total phenolic acids in plant-derived foods by HPLC with diode-array detection. *Journal of Agricultural and Food Chemistry*, 50(13), 3660-3667. <http://dx.doi.org/10.1021/jf020028p>. PMID:12059140.
- Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E., & McLaughlin, J. L. (1982). Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Medica*, 45(5), 31-34. <http://dx.doi.org/10.1055/s-2007-971236>.
- Mishra, B. K., Rastogi, A., & Shukla, S. (2012). Regulatory role of mineral elements in the metabolism of medicinal plants. *Medicinal and Aromatic Plant Science and Biotechnology*, 6, 1-23.
- Moein, M. R., Moein, S., & Ahmadi-zadeh, S. (2008). Radical scavenging and reducing power of *Salvia mirzayanii* subfractions. *Molecules*, 13(11), 2804-2813. <http://dx.doi.org/10.3390/molecules13112804>. PMID:19015620.
- Mohan, S. C., Balamurugan, V., Salini, S. T., & Rekha, R. (2012). Metal ion chelating activity and hydrogen peroxide scavenging activity of medicinal plant *Kalanchoe pinnata*. *Journal of Chemical and Pharmaceutical Research*, 4, 197-202.
- Murakami, A., Ashida, H., & Terao, J. (2008). Multitargeted cancer prevention by quercetin. *Cancer Letters*, 269(2), 315-325. <http://dx.doi.org/10.1016/j.canlet.2008.03.046>. PMID:18467024.
- Neuhouser, M. L. (2004). Dietary flavonoids and cancer risk: evidence from human population studies. *Nutrition and Cancer*, 50(1), 1-7. http://dx.doi.org/10.1207/s15327914nc5001_1. PMID:15572291.
- Nguta, J. M., Mbaria, J. M., Gakuya, D. W., Gathumbi, P. K., Kabasa, J. D., & Kiama, S. G. (2012). Evaluation of acute toxicity of crude plant extracts from Kenyan biodiversity using brine shrimp, *Artemia salina* L. (Artemiidae). *The Open Conference Proceedings Journal*, 3(1), 30-34. <http://dx.doi.org/10.2174/2210289201203010030>.
- Ogbiko, C., Usman, D., Eboka, J., & Igbe, I. (2017). Anti-Ulcer activity of methanol extract of *Plantago rugelii* Decne. (Plantaginaceae). *Tropical Journal of Natural Product Research*, 1(2), 84-88. <http://dx.doi.org/10.26538/tjnpr/v1i2.7>.
- Oliszowy, M., & Dawidowicz, A. (2018). Is it possible to use the DPPH and ABTS methods for reliable estimation of antioxidant power of colored compounds? *Chemical Papers*, 72(2), 393-400. <http://dx.doi.org/10.1007/s11696-017-0288-3>.
- Pérez-Jiménez, J., & Saura-Calixto, F. (2006). Effect of solvent and certain food constituents on different antioxidant capacity assays. *Food Research International*, 39(7), 791-800. <http://dx.doi.org/10.1016/j.foodres.2006.02.003>.
- Phaniendra, A., Jestadi, D. B., & Periyasamy, L. (2015). Free radicals: Properties, sources, targets, and their implication in various diseases. *Indian Journal of Clinical Journal of Biochemistry*, 30(1), 11-26. <http://dx.doi.org/10.1007/s12291-014-0446-0>. PMID:25646037.

- Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical Biochemistry*, 269(2), 337-341. <http://dx.doi.org/10.1006/abio.1999.4019>. PMID:10222007.
- Rufino, M. S. M., Alves, R. E., de Brito, E. S., Pérez-Jiménez, J., Saura-Calixto, F., & Mancini-Filho, J. (2010). Bioactive compounds and antioxidant capacities of 18 nontraditional tropical fruits from Brazil. *Food Chemistry*, 121(4), 996-1002. <http://dx.doi.org/10.1016/j.foodchem.2010.01.037>.
- Santos, M. C. P., & Gonçalves, E. C. B. A. (2016). Effect of different extracting solvents on antioxidant activity and phenolic compounds of a fruit and vegetable residue flour. *Scientia Agropecuaria*, 7(1), 7-14. <http://dx.doi.org/10.17268/sci.agropecu.2016.01.01>.
- Shabir, G., Anwar, F., Sultana, B., Khalid, Z. M., Afzal, M., Khan, Q. M., & Ashrafuzzaman, M. (2011). Antioxidant and antimicrobial attributes and phenolics of different solvent extracts from leaves, flowers and bark of Gold Mohar [*Delonix regia* (Bojer ex Hook.) Raf.]. *Molecules*, 16(9), 7302-7319. <http://dx.doi.org/10.3390/molecules16097302>. PMID:22143540.
- Shankar, S., Singh, G., & Srivastava, R. K. (2007). Chemoprevention by resveratrol: molecular mechanisms and therapeutic potential. *Frontiers in Bioscience*, 12(12), 4839-4854. <http://dx.doi.org/10.2741/2432>. PMID:17569614.
- Silva, A. M., Santos, C. M., Cavaleiro, J. A., Tavares, H. R., Borges, F., & Silva, F. A. (2001). NMR studies on the antiradical mechanism of phenolic compounds towards 2,2-diphenyl-1-picrylhydrazyl radical. In G. A. Webb, P. S. Belton, A. M. Gil & I. Delgado (Eds.), *Magnetic resonance in food science - a view to the future* (8th ed., pp. 110-116). London: Royal Society Chemistry.
- Slinkard, K., & Singleton, V. L. (1977). Total phenol analysis: automation and comparison with manual methods. *American Journal of Enology and Viticulture*, 28, 49-55.
- Stankovic, M. S., Niciforovic, N., Topuzovic, M., & Solujic, S. (2011). Total phenolic content, flavonoid concentrations and antioxidant activity, of the whole plant and plant parts extracts from *Teucrium montanum* L. var. *montanum*, *F. supinum* (L.) Reichenb. *Biotechnology, Biotechnological Equipment*, 25(1), 2222-2227. <http://dx.doi.org/10.5504/BBEQ.2011.0020>.
- Sultana, B., Anwar, F., & Ashraf, M. (2009). Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules*, 14(6), 2167-2180. <http://dx.doi.org/10.3390/molecules14062167>. PMID:19553890.
- Talukder, M. E. U., Aklima, J., Bin Emran, T., Islam, S., Rahman, A., & Bhuiyan, R. H. (2013). *In vitro* antioxidant potential of *Momordica charantia* fruit extracts. *British Journal of Pharmaceutical Research*, 3(4), 963-971. <http://dx.doi.org/10.9734/BJPR/2013/4722>.
- Tee-ngam, P., Nunant, N., Rattanasat, P., Siangproh, W., & Chailapakul, O. (2013). Simple and rapid determination of ferulic acid levels in food and cosmetic samples using paper-based platforms. *Sensors*, 13(10), 13039-13053. <http://dx.doi.org/10.3390/s131013039>. PMID:24077320.
- Tepe, B., Daferera, D., Tepe, A. S., Polissiou, M., & Sokmen, A. (2007). Antioxidant activity of the essential oil and various extracts of *Nepeta flavida* Hub.-Mor. from Turkey. *Food Chemistry*, 103(4), 1358-1364. <http://dx.doi.org/10.1016/j.foodchem.2006.10.049>.
- Tomescu, A., Rus, C., Pop, G., Alexa, E., Radulov, I., Imbrea, I. M., & Negrea, M. (2015). Researches regarding proximate and selected composition of some medicinal plants belonging to the Lamiaceae family. *Lucrari Stiintifice Seria Agronomie Agronomie*, 58, 175-179.
- Tungmunnithum, D., Thongboonyou, A., Pholboon, A., & Yangsabai, A. (2018). Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medicinal aspects: an overview. *Medicines*, 5(3), 93. <http://dx.doi.org/10.3390/medicines5030093>. PMID:30149600.
- Turkmen, N., Sari, F., & Velioglu, Y. S. (2006). Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin-Ciocalteu methods. *Food Chemistry*, 99(4), 835-841. <http://dx.doi.org/10.1016/j.foodchem.2005.08.034>.
- Tusevski, O., Kostovska, A., Iloska, A., Trajkovska, L., & Simic, S. G. (2014). Phenolic production and antioxidant properties of some Macedonian medicinal plants. *Central European Journal of Biology*, 9, 888-900.
- Velasco-Reynold, C., Navarro-Alarcon, M., López-GaDe La Serrana, H., & Lopez-Martinez, M. C. (2008). Copper in foods, beverages and waters from South East Spain: influencing factors and daily dietary intake by the Andalusian population. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 25(8), 937-945. <http://dx.doi.org/10.1080/02652030801984117>. PMID:18608494.
- Vlase, L., Benedec, D., Hanganu, D., Damian, G., Csillag, I., Sevastre, B., Mot, A. C., Silaghi-Dumitrescu, R., & Tilea, I. (2014). Evaluation of antioxidant and antimicrobial activities and phenolic profile for *Hyssopus officinalis*, *Ocimum basilicum* and *Teucrium chamaedrys*. *Molecules*, 19(5), 5490-5507. <http://dx.doi.org/10.3390/molecules19055490>. PMID:24786688.
- Yang, J., Guo, J., & Yuan, J. (2008). *In vitro* antioxidant properties of rutin. *Lebensmittel-Wissenschaft + Technologie*, 41(6), 1060-1066. <http://dx.doi.org/10.1016/j.lwt.2007.06.010>.
- Zduńska, K., Dana, A., Kolodziejczak, A., & Rotsztein, H. (2018). Antioxidant properties of ferulic acid and its possible application. *Skin Pharmacology and Physiology*, 31(6), 332-336. <http://dx.doi.org/10.1159/000491755>. PMID:30235459.