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ANTIOXIDANT ACTIVITY AND MINERAL INGREDIENT ASSESSMENT OF DIFFERENT SOLVENT EXTRACTS OF *PARONYCHIA CHIONAEA*

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Abstract

The present study was conducted to determine total phenolic content, DPPH free radical scavenging properties, total antioxidant/oxidant status and mineral substance content of methanol and acetone extracts of endemic *Paronychia chionaea* species. The findings indicated that the phenolic substance content was higher in acetone extract (112.76 ± 8.4 µg GAE / mg extract) when compared to the methanol extract (98.72 ± 6.06 µg GAE / mg extract). It was observed that the radical scavenging effect of *Paronychia chionaea*methanol extract (70.5%) was similar to that of the synthetic antioxidant BHT (74.8%) and higher than the effects of the acetone extract (59.8%). The total antioxidant and oxidant status of the *Paronychia chionaea*methanol extract were 2.84 ± 0.3 µmolTroloxEq/g and 6.56 ± 0.8 µmol H_2O_2Eq/g, respectively and the total antioxidant and oxidant status of acetone extract were 2.68 ± 0.4 µmolTroloxEq/g and 6.22 ± 0.6 µmol H_2O_2Eq/g, respectively. Due to its Mn, Fe, and Cu content, which are minerals that are incorporated in the enzyme structure, its total phenolic substance levels, its antiradical properties and antioxidant/oxidant status, *Paronychia chionaea*was with high bio-value. The present study is considered to provide information for future studies.

Keywords: *Paronychia chionaea,* DPPH scavenging activity, Total oxidant/antioxidant status, Total phenolic content, Bioelement

Introduction

Paronychia is a genus of the family Caryophyllaceae. The genus is represented by 28 species in Turkey out of which 20 are endemic. This genus is indigenous in Irano-Turanien and East-Mediterranean region (Kaplan, 2008; Davis, 1967).

Paronychia chionaea. is an endemic species described by Pierre Edmond Boissier in 1843. It is a perennial, herbaceous species with evergreen leaves. It grows in rocky habitats in western and central Anatolia elevation of 1100-2800 m. In a phytochemical study conducted with *Paronychia chionaea* secondary metabolite isolation was conducted on *Paronychia chionaea* leaves. This study revealed that the species contained two secondary metabolites; 6-C-[alpha-L-arabinopyranosyl-(1-->2)-beta-D-glucopyranosyl]-7-O-[beta-D-glucopyranosyl]-luteolin 3'-methyl ether and 2-(methoxy)-2-(3,5-dimethoxy 4-hydroxyphenyl)-ethane-1,2-diol 1-O-beta-D-glucopyranoside (Avunduk *et al.,* 2011).

In another study, 4 new triterpenoidsaponins, chionaeosides A-D (1-4) were isolated from the roots of *Paronychia chionaea*:3-o-α-L-arabinopyranosylgypsogenicacid 28-*O*-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside(**1**), 3-*O*-α-L-arabinopyranosylgypsogenicacid 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (**2**),3-*O*-α-L-arabino pyranosylgypsogenic acid 28-*O*-β-D-glucopyranoside (**3**), and 3-*O*-α-L-arabino pyranosylgypsogenic acid (**4**) (Avunduk *et al.,* 2007).

Secondary metabolites are the products of the mechanisms that are produced by plants and are not directly related to the basic vital functions of the plant, but necessary to maintain defense, protection, adaption, survival and sustaining future generations in plants. The main secondary metabolites are phenolic compounds, terpenes and alkaloids. All plants contain phenolic compounds in different qualities and quantities. Phenolic compounds, in plants, are the most common group of substances in plants and newly identified phenolic substances, are added to the list. The phenolic compounds that the plants contain protect the cells against oxidative damage induced by reactive oxygen species or free radicals. (Shoib & Shahid, 2015; Penny *et al.,* 2002)

The present study was conducted to determine the total phenolic content of methanol and acetone extracts of the endemic *Paronychia chionaea*species. Furthermore, the study aimed to identify free radical scavenging properties, total oxidant status, total antioxidant status and mineral substance content in methanol and acetone extracts of *Paronychia chionaea* species.

Material and Method

Plant material: The *Paronychia chionaea* species was collected in Ulupinar village, Banaz, Usak province in Turkey at an altitude of 1296 m on 15.09.2015, (36 S 0272059, UTM 426187515). Collected plants were identified by Dr. Mustafa Kargıoğlu, and stored at AfyonKocatepe University, Faculty of Sciences and Literature Herbarium.

Preparation of the plant and analyses: A mixture of *Paronychia chionaea* root, stem, leaves and flowers was used for plant extracts. These plant sections were broken into pieces and the product was dried in the shade at ambient temperature. To prepare the extracts, methanol and acetone were added on the pulverized *Paronychia chionaea.* Free radical scavenging activity and total phenolic content were determined using the prepared extracts (Gülçin, 2005).

The total phenolic content was determined with the Folin-Ciocalteu method. Folin-Ciocalteu reagent and Na_2CO_3 were added to *Paronychia chionaea* methanol and acetone extract solutions. The absorbance of the mixture was measured at 760 nm against water. The results were calculated with the Gallic acid calibration curve, which on the standard method for indicating Gallic acid equivalence (Slinkard& Singleton, 1977).

DPPH$^{\bullet}$ (2,2-diphenyl-1-picryl hydrazine) was used as a free radical to determine the free radical scavenging activities of methanol and acetone extracts of *Paronychia chionaea* extracts. Depending on the antiradicals in the extracts, DPPH causes the reduction of the purple color and the absorbance. Ethanol and DPPH solution were added to the samples with different concentrations. After incubated in dark, the absorbance of the samples was measured (Blois, 1958).

To determine the total antioxidant status and total oxidant status, dissolvent was added to the sections obtained from the dried and pulverized plants and the samples were sonicated. The samples were filtered through the filter paper and centrifuged. The supernatant was removed and the samples were centrifuged again and used in the analyses. Total antioxidant/oxidant status (TAS/TOS) were measured with commercial kits. The TAS kit procedure is based on the principle of ABTS$^{\cdot}$radical formation and loss of the initial color in the radical based on the antioxidant content of the plant. The intensity of the color decreases based on the antioxidant content and capacity. Absorbance was measured with a spectrophotometer. The TOS kit procedure is based on the oxidization of Fe^{+2} complexes into Fe^{+3} complex by the oxidants in the plant. The Fe^{+3} produced by the plant oxidants forms a colored complex with xylenol orange. Color intensity varies with the sample oxidant content. Absorbance was measured with a spectrophotometer. Oxidative stress index (OSI) was calculated by dividing the total oxidant status (TOS) by the total antioxidant status (TAS) (Dıkılıtas *et al.*, 2011).

To determine the minerals content of the species, nitric acid, hydrogen peroxide and perchloric acid were added to the dried and pulverized plant section and the organic components were deformed in the microwave and utilized in the analysis. Samples were kept in the microwave oven at 90-150°C for certain periods. The bioelement concentrations were measured with inductively coupled plasma-optical emission spectroscopy (ICP-OES Spectro Genesis, Germany) (Aksoy *et al.*, 2016).

Statistical Analyses

The mean of four separate analysis results were presented as analysis findings. Data analysis was conducted with SPSS (version 15 for Windows, SPSS Inc.) software and findings were recorded as mean ± standard deviation.

Results and Discussion

Phenolic compounds found in plants are water-soluble substances. They are divided in two groupsnamely phenolic acids and flavonoids. Phenolic compounds contain aromatic rings in addition to at least one -OH group in their structure. They can perform radical scavenging and metal chelating functions. The antioxidant activity of the phenolic compounds is related to the number of hydroxyl groups in their structure and the position of the hydroxyl group. They are molecules with antioxidant properties due to these structures. The phenolic compounds in the plant protect the cell against oxidative damage induced by reactive oxygen species or free radicals (Ghasemzadeh&Ghasemzadeh, 2011; Shoib & Shahid, 2015; Penny *et al*., 2002; Ashraf *et al*., 2015).

The total phenolic compounds content in the methanol and acetone extracts obtained from the root and stems of *Paronychia chionaea* species was determined. For this purpose, the Gallic acid, the standard phenolic compound, was used and the graphs were plotted. The total phenolic compounds content in both extracts was calculated as Gallic acid equivalent (GAE) using the formula obtained with the standard graphic (r^2: 0.9908). The standard Gallic acid graph constructed for this purpose is presented in Fig. 1.

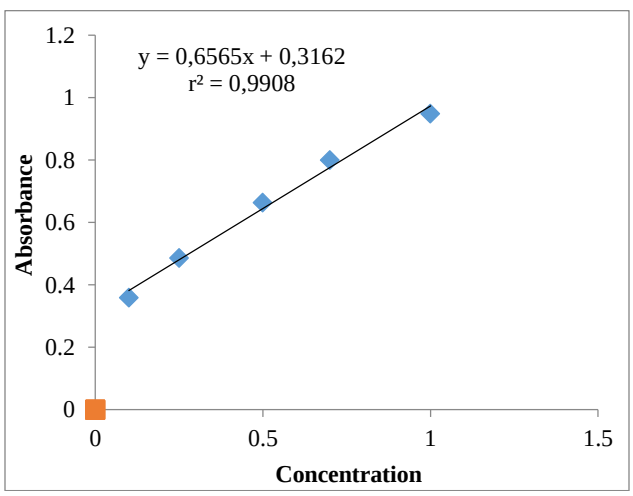

Fig. 1. Gallic acid standard curve.

Table 1. Total phenolic compound content in 1 mg *Paronychia chionaea* methanol and acetone extracts.

	PCM	PCA
Total phenolic compound µG GAE/mg ekstre	98.72±6.06	112.76±8.4

The results are given as mean ± SD (n = 4). PCM: *Paronychia chionaea* methanol extract; PCA: *Paronychia chionaea* acetone extract

The total phenolic compound content in 1 mg methanol and acetone extracts that were obtained from the root and stem sections of *Paronychia chionaea* plant is presented in Table. 1

The phenolic compound content found in 1 mg *Paronychia chionaea* plant methanol and acetone extracts were 98.72 ± 6.06 and 112.76 ±8.4 µg GAE, respectively. It was observed that the solvents used to determine the phenolic compounds in the plant and the resulting antioxidant activity showed different results. It was found that the acetone extract of the plant had higher phenolic compound content when compared to the methanol extract. This finding also demonstrated that selection of an adequate solvent would yield better phenolic content in the plant, thus providing a higher antioxidant capacity.

Although DPPH is not a biological radical, it is a macrobiotic nitrogen-based marker, which is used for the determination of free radical scavenging activities of antioxidants. It is commonly used to determine the radical scavenging activities of compounds that contain antioxidants. When the purple colored solution interacts with an antioxidant compound, it is reduced to the yellow colored diphenylpicryl hydrazine. In this method, the DPPH radical is reduced by antioxidant substances with hydrogen donor groups (Enujiugha *et al.*, 2012).The graphs on the radical scavenging capacities of plant extracts used in the study are presented in Fig. 2-4. In the study, a standard graph was generated for BHT, a synthetic antioxidant compound (r^2 = 0.9895)

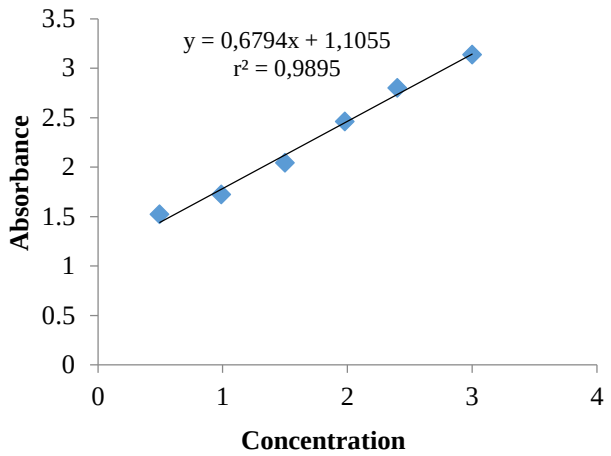

Fig. 2. Standard DPPH graph prepared for DPPH radical scavenging activity determination

The DPPH radical scavenging activities of methanol and acetone extracts of *Paronychia chionaea* plant were determined as shown in Fig. 2.

Absorbance = 0.6794 [DPPH] + 1.1055

The DPPH radical scavenging activities of methanol and acetone extracts of *Paronychia chionaea* plant are presented in Fig. 3. It could be observed in the Fig. 3 that extracts DPPH radical scavenging activities increased with the increase in concentration.

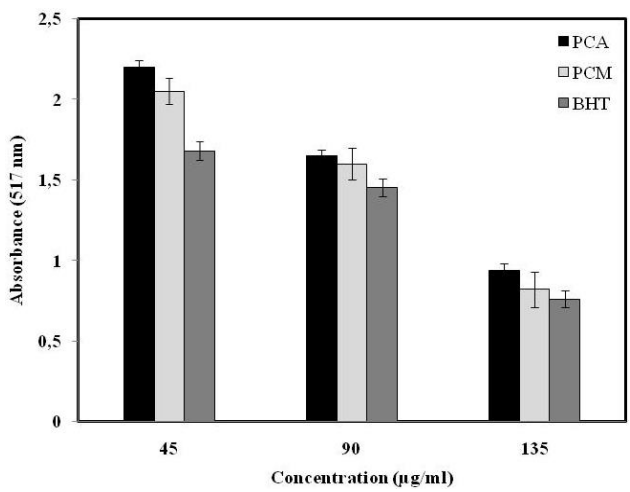

Fig. 3. Comparison of DPPH radical scavenging activity in different concentrations (45-135 µg/mL) with BHT.

The DPPH radical scavenging activities of methanol and acetone extracts obtained from *Paronychia chionaea* plant and 135 µg/mL standard antioxidant BHT were determined as follows: BHT ~ *Paronychia chionaea* methanol extract>*Paronychia chionaea* acetone extract. It was found that DPPH removal activities of methanol and acetone extracts of *Paronychia chionaea* plant was 74.8% in BHT, 70.5% in methanol extract and 59.8% in acetone extract. Results demonstrated that especially *Paronychia chionaea* methanol extract exhibited similar findings with BHT, which is a synthetic antioxidant.

The plants are exposed to stress due to various environmental factors. Fungi, bacteria and virus infections and the harmful animals are attacked by the resulting biotic stress. Water, temperature, radiation, chemicals, magnetic and electrical fields create abiotic stress. In existing stress plants endogenously in photosynthesis reactions in chloroplasts, in plastids and peroxisomes, NADPH oxidase in the citric acid cycle in mitochondria causes ROS formation by the action of enzymes such as cell wall peroxidases and amino oxidases. The plants have both enzymatic and non-enzymatic defense systems against the ROS. Enzymatic defense includes enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GPx). Non-enzymatic defense takes place via phytochemicals found in plants such as ascorbic acid, glutathione, phenolic compounds and aminoacids. The measurement of the total antioxidant capacity provides more valuable information when compared to individual measurement of antioxidants. Measuring individual antioxidants requires time consuming, expensive, and complex techniques. Thus, total antioxidant capacity or total antioxidant status measurement is the currently preferred and widely used method (Weydert&Cullen, 2010; Szymonik-Lesiuk *et al.*, 2003; Zhan *et al.*, 2004; Abideen *et al.*, 2015). Total antioxidant status. Oxidative stress index (TOS/TAS) data for the methanol and acetone extracts of *Paronychia chionaea* plant roots and stems are presented in Tab. 2.

Table 2. Total antioxidant status, total oxidant status and oxidative stress index of *Paronychia chionaea*.

	PCM	PCA
TAS, µmolTroloxEq/g	2.84 ± 0.3	2.68 ± 0.4
TOS, µmol H_2O_2Eq/g	6.56 ± 0.8	6.22 ± 0.6
OSI, Arbitrary Unit	3.72 ± 0.2	2.32 ± 0.3

The results are given as mean ± SD (n = 4). PCM: *Paronychia chionaea* methanol extract PCA: *Paronychia chionaea* acetone extract; TAS: Total antioxidant status; TOS: Total oxidant status; OSI: Oxidative stress index (TOS / TAS)

Several studies (Kalt*et al.*, 1999; Cao *et al.*, 1996) were conducted to determine the antioxidant capacities of vegetables and fruits. The total antioxidant capacity of *Paronychia chionaea* was lower than that of garlic, onion, plum and strawberry (19.4, 4.5, 9.49, 15.36 µmolTroloxEq/g, respectively). However, higher than species such as tomato, carrot, banana and pear, which are known for their antioxidant properties (2.1, 1.89, 2.21, 1.34 µmolTroloxEq/g, respectively).

Plants' defenses against ROS that are formed due to negative environmental conditions such as drought, air pollution, nutrient deficiency, salinity and heat include enzymatic defense systems in chloroplasts, mitochondria, peroxisomes and the cytoplasm. SOD, CAT and GPx are the most important enzymatic antioxidants. These three enzymes are metallo-enzymes that contain elements in their structures. SOD is an effective metallo-enzyme responsible for the defense against ROS in all organisms with aerobic respiratory systems. These are antioxidant enzymes that are bound to metal cofactors such as Cu/Zn-SOD, Mn-SOD and Fe-SOD. CAT is an enzyme with tetrameric Fe that is found in peroxisomes and responsible for the detoxification of H_2O_2. GPx plays a role in the reduction of H_2O_2, organic hydro-peroxides and lipid peroxides using glutathione. It is a tetrameric enzyme with four selenium (Se) atoms (Bogdanska *et al.,* 2003;Caverzan *et al.,* 2016; Racchi, 2013). Bio-element levels found in the mixture obtained from the stem and root sections of the *Paronychia chionaea* plant are presented in Tab. 3.

Table 3. Mineral levels in *Paronychia chionaea* plant.

Element	Concentration (ppm)	Element	Concentration (ppm)
Al	1653.64±120.16	Ga	2.871±0.893
B	9.602±1.83	K	1876.63±117.65
Ba	15.024±1.52	Co	0.050±0.011
Be	0.043±0.02	Li	1.237±0.28
Bi	3.983±0.68	Mg	872.57±35.96
Ca	3522.71±52.8	Mn	83.423±13.75
Cr	3.650±1.06	Na	136.649±13.45
Cu	5.814±1.265	Ni	4.935±1.06
Fe	1847.43±14.56	Pb	2.835±0.8

The results are given as mean ± SD (n = 4)

As seen in the table, the elements of Be and Co, which are not found in many species, were observed in this species. It was found that the mineral substances identified as antioxidants were found in sufficient amounts in the *Paronychia chionaea*plant. Since the selenium concentration was not within detectable limits, the level of this element was not determined. However, the presence of other antioxidant enzymes, namely Cu, Zn and Mn in SOD and Fe concentrations in CAT, demonstrated that the species could possess antioxidant properties.

Conclusion

The phenolic content of *Paronychia chionaea*, the fact that its radical scavenging effect was close to that of the antioxidant BHT, its TAS was higher when compared to plant species known as antioxidants and it contained the elements that participated in the antioxidant enzyme structure showing that the species had radical scavenging properties and antioxidant capacity. It was observed that the plant methanol extract had antiradical properties, while the acetone extract had high phenolic substance content and antioxidant capacity. The present study will contribute to the literature since it was the first study where the antioxidant activity of endemic *Paronychia chionaea* species was determined. It was considered that future studies in phytotherapy should evaluate *Paronychia chionaea* even further.

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