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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Chemical Properties and Antioxidant Activity of Different Extracts from Purslane (*Portulaca Oleracea* L.)*

Semizotunun (*Portulaca Oleracea* L.) Farklı Ekstraktlarının Kimyasal Özellikleri ve Antioksidan Aktivitesi

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Abstract

Purslane (*Portulaca oleracea* L.) has been known as a medical herb with valuable nutritional and pharmacological properties. Herein, water, methanol, and acetone extracts were prepared from two morphologically different purslane samples, thereafter their content of β-carotene, chlorophyll a and b, total phenolic compounds (TPC), total flavonoids, and total flavonols was evaluated. Additionally, DPPH• scavenging activity and ferrous iron (Fe^{+2}) chelating activity of these extracts were also measured. The acetone extract had the highest amounts of β-carotene (14.16 mg g^{-1} DW), chlorophyll a (40.46 mg g^{-1} DW), chlorophyll b (9.94 mg g^{-1} DW), TPC (51.01 mg GAE g^{-1} DW), flavonoids (133.23 mg RE g^{-1} DW), and flavonols (46.94 mg RE g^{-1} DW). Whereas the lowest values were observed in the water extract, as 0.03 mg g^{-1} DW, 0.03 mg g^{-1} DW, 0.06 mg g^{-1} DW, (34.24 mg GAE g^{-1} DW, 10.98 mg RE g^{-1} DW, 3.28 mg RE g^{-1} DW, respectively. The acetone extract also showed higher DPPH• scavenging activity (IC_{50} = 57.23 µg mL^{-1}) compared to methanol (IC_{50} = 65.80 µg mL^{-1}) and water (IC_{50} = 71.47 µg mL^{-1}) extracts. Regarding the ferrous iron (Fe^{+2}) chelating activity, methanol extract exhibited the highest value followed by water and acetone extracts, being 12.78, 10.20 and 6.08%, respectively. A positive correlation was detected between the DPPH• scavenging activities of the purslane extracts and their content of TPC, flavonoid, flavonol, β-carotene, chlorophyll a, and chlorophyll b. On the other hand, there was no significant correlation between chelating activity of the extracts and their content of β-carotene, chlorophyll a, TPC, flavonoids, flavonols, and DPPH scavenging activity. Purslane extracts could be suggested as a natural antioxidant substance for many pharmaceutical and food applications.

Keywords: *Portulaca oleracea*, Purslane extract, DPPH• scavenging activity, Phenolic compound, Metal chelating

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Öz

Semizotu (*Portulaca oleracea* L.), besinsel değeri ve farmakolojik özellikleri ile tıbbi bir bitki olarak bilinmektedir. Çalışmada, morfolojik olarak farklı özelliklere sahip iki semizotu örneğinin su, metanol ve aseton kullanılarak üç farklı çözücü ile ekstraktları elde edilmiştir. Elde edilen ekstraktların β-karoten, klorofil a ve b, toplam fenolik madde (TFM), toplam flavonoid ve toplam flavonol içerikleri belirlenmişe olarak bu ekstraktların DPPH• radikal giderme aktivitesi ve Ferrous iron (Fe^{+2}) çelatlama aktivitesi de ölçülmüştür. Aseton ekstraktının en yüksek β-karoten (14.16 mg g^{-1} DW), klorofil a (40.46 mg g^{-1} DW), klorofil b (9.94 mg g^{-1} DW), total fenolik madde (51.01 mg GAE g^{-1} DW), flavonoid (133.23 mg RE g^{-1} DW) ve flavonol (46.94 mg RE g^{-1} DW) içeriğine sahip olduğu tespit edilmiştir. En düşük değerleri ise, sırasıyla 0.03 mg g^{-1} DW, 0.03 mg g^{-1} DW, 0.06 mg g^{-1} DW, 34.24 mg GAE g^{-1} DW, 10.98 mg REg^{-1} DW, 3.28 mg RE g^{-1} DW su ekstraktında bulunmuştur. Ayrıca aseton ekstraktının (IC_{50} = 57.23 µg mL^{-1}) metanol (IC_{50} = 65.80 µg mL^{-1}) ve su (IC_{50} = 71.47 µg mL^{-1}) ekstraktlarına kıyasla daha yüksek DPPH• giderme aktivitesi gösterdiği belirlenmiştir. Ferro demir (Fe^{+2}) çelatlama aktivitesi bakımından, metanol ekstraktı (%12.78) en yüksek ferro demir (Fe^{+2}) çelatlama aktivitesi göstermiş, bunu sırası ile su ekstraktı (%10.20) ve aseton ekstraktı (%6.08) takip etmiştir. Semizotu ekstraktlarının DPPH• giderme aktivitesi ile total fenolik madde, flavonoid, flavonol, β-karoten, klorofil a ve klorofil b içerikleri arasında pozitif bir korelasyon izlenmiştir. Öte yandan, ekstraktların ferro demir (Fe^{+2}) çelatlama aktivitesi ile β-karoten, klorofil a, TFM, flavonoid ve flavonol içerikleri ve DPPH• giderme aktivitesi arasında önemli bir korelasyon tespit edilmemiştir. Semizotu ekstraktlarının çeşitli farmasötik ve gıda sanayiinde doğal antioksidan madde olarak kullanılması önerilebilir.

Anahtar Kelimeler: *Portulaca oleracea*, Semizotu ekstraktı, DPPH• giderme aktivitesi, Fenolik bileşikler, Çelatlama aktivitesi

1. Introduction

Fruit, vegetables, and herbs are recognized as good sources of natural bioactive compounds which exhibit a wide range of nutritional and pharmacological properties. These compounds, particularly polyphenols, carotenoids, glucosinolates, and vitamins (vitamin C and tocopherol) have been demonstrated to represent high antioxidant activity and consequently protect and/or reduce the risk of many chronic diseases due to their ability to reduce oxidative stress and prevent oxidation of macromolecules (Barba et al., 2014; Shashirekha et al., 2015; Xu et al., 2017; Kök et al., 2018; Binici and Şat, 2021; Tahmaz et al., 2022). In response to the consumers' increased demand for more natural products, the natural antioxidants extracted from plants have been promoted as pharmaceuticals, cosmetic ingredients, and food additives, being safer, healthier, and causing fewer adverse responses compared to synthetic antioxidants such as BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), and TBHQ (tert-Butylhydroquinone) (Tadhani et al., 2007; Barba et al., 2014; Ünal, 2017; Xu et al., 2017).

Purslane (*Portulaca oleracea* L.), belonging to the family *Portulacaceae*, is an annual cosmopolitan herb. Because of its extreme adaptability and tolerance to severe conditions, purslane is widely spread all over the world, especially in tropical and subtropical areas (Uddin et al., 2012b; Alam et al., 2014b). Purslane leaves and stems are usually consumed fresh in various salads. However, fresh and dried purslane could be also added to various meals, soups, and even consumed as pickles. In addition, it could be used as a food additive (emulsifier or thickener) and as a natural ingredient in skincare products (Poeydomenge and Savage, 2007; Alam et al., 2014a; Binici et al., 2021).

Besides its usage in the human diet, purslane has been commonly used for medical purposes by various ancient cultures, being familiar as “a vegetable for long life” in Chinese culture. Recently, several health benefits have been confirmed by in vitro and in vivo research, experimental animals, and clinical studies. Due to its nutritional and pharmacological properties, World Health Organization (WHO) has stated common purslane as one of the most used medicinal plants and recognized it as a ‘‘global panacea”. Common purslane and its extracts are reported to exhibit antispasmodic, antiarthritic, antiseptic, antimicrobial, antifungal, anticancer, antidiabetic, and antimutagenic effects (Cho et al., 2008; YouGuo et al., 2009; Hasssan, 2014; Silva and Carvalho, 2014). They also play a valuable role in inhibiting some chronic diseases such as cardiovascular, and neurodegenerative (Dkhil et al., 2011).

A diverse group of valuable ingredients have been reported in purslane and/or its extracts including polyphenols, vitamins (particularly vitamins A and C), minerals (particularly potassium, calcium, and magnesium), and water-soluble polysaccharides. Several bioactive compounds principally glutathione, noradrenaline, dopamine β-carotene, and α-tocopherol were also documented (Uddin et al., 2014; Petropoulos et al., 2015; Binici et al., 2021). Various alkaloids such as nandigerine, angustureine, reticuline, itingensine, homolycorine were also identified in purslane (Aziz et al., 2016). Polyunsaturated fatty acids are the major fatty acid group in common purslane. Additionally, purslane is considered one of the richest vegetable sources of the essential fatty acid (alpha-linolenic acid (ALA), C18:3, n-3) accounting for about 60% of the total fatty acid content in leaves and 40% in seeds (Liu et al., 2000; Petropoulos et al., 2015). Furthermore, a remarkably higher level of melatonin (19000 pg g^{-1}) was detected in fresh purslane leaves compared to many other fruits and vegetables (Simopoulos et al., 2005). Other important chemical compounds found in significant amounts in purslane are flavonoids (particularly quercetin, rutin, and myricetin) and phenolic acids (particularly chlorogenic, rosmarinic, and E-vanillic acids) suggesting purslane and its extracts as an important source of antioxidants (Siriamornpun and Suttajit, 2010; Hassan, 2014; Sallam et al., 2017).

Many authors suggested methanol as the most suitable solvent for extracting phenolic compounds from purslane (Uddin et al., 2012a; Sallam et al., 2017). In contrast, Cai et al. (2004) stated higher levels of total phenolics when hot water was used as a solvent compared to methanol. In other study, acetone extracts were reported to possess higher levels of total phenolic content and antioxidant activity compared to methanol and water extracts (Güngören et al., 2017). The phenolic content of purslane and consequently its antioxidant activity is strongly affected by various factors including species origin, variety, plant maturity, and harvesting conditions (Alam et al., 2014a; Uddin et al., 2012a); as well as the extraction method and technique, used solvent, extraction temperature, and time (Alam et al., 2014a; Güngören et al., 2017; Sallam et al., 2017; Uddin et al., 2012a).

Therefore, a wide variation is seen in the literature related to the phenolic contents of purslane and its antioxidant activity.

There is limited data related to the chemical composition and antioxidant activity/capacity of purslane that is grown in Turkey. Moreover, there is inconsistency in the information about the most appropriate solvent for extracting a higher portion of phenolic compounds. Thus, the aim of this study was to identify the most effective solvent for the extraction of bioactive compounds from purslane. For this aim, the antioxidant activity and some chemical properties including the contents of fat-soluble pigments, total phenolic compounds, flavonoids, and flavonols of various purslane extracts (water, methanol, and acetone) were measured from two morphologically different samples.

2. Materials and Methods

2.1. Plant materials

Two morphologically different samples of purslane (*Portulaca oleracea* L.) were obtained from the domestic market of Erzurum-Turkey. The first sample (Sample-1) had large, green (dark), fleshy leaves, and thick stems, whereas the other sample (Sample-2) had small, yellow-green (chartreuse color), and thin leaves. The stems of this sample were thinner compared to Sample-1 and their lower bits were red in color. Each sample was cleaned thoroughly, cut into small pieces, and frozen at – 20°C. The frozen samples were freeze dried (lyophilized) at – 80°C for 36 h with Operon (FDU-8612, KR) lyophilizer. Then the dried samples were ground, homogenized with a high-speed blender (Waring, 8011ES), and kept in glass jars at 4°C until the extraction.

2.2. Purslane extracts preparation

Purslane extracts were prepared by maceration of each sample with distilled water, methanol, and acetone according to the procedures described by Alam et al. (2014a) with some changes. 10 g of lyophilized purslane powder was mixed with 150 mL of solvent (1:15, g mL^{-1}) in dim light at ambient temperature for about 14 h using an orbital shaker (Biocote-SSL1, UK). The mixture was then passed through filter paper (Whatman No:1) and the plant's remnant was reextracted with fresh solvent for 3 h following the same steps mentioned previously. A rotary evaporator (Heidolph laborata 4000 efficient- HB digital) was used to concentrate the supernatants and the final volumes were reduced to 70 mL. The concentrated supernatants were evaporated at 45°C using a vacuum oven (WiseVen, WOV-30). Obtained extracts were stored at – 20 °C in screw cap glass brown bottles until further analyses. The dried extracts were redissolved in methanol at 2000 µg mL^{-1} (stock solution) immediately prior to the analyses.

2.3. Fat-soluble pigments (β-carotene and chlorophyll a and b) contents

Estimation of β-carotene and chlorophylls a and b was carried out according to the procedures represented by Barros et al. (2011) as follows: 100 mg of the purslane dried extract was thoroughly mixed with 10 mL of the acetone-hexane mixture (4:6) for 5 min. The filtrates (obtained by filtration through Whatman, No:1) were then diluted with the acetone-hexane mixture and their absorbances were measured at three different wavelengths (453, 505, 645, and 663 nm) against blank. The amounts of the fat-soluble pigments were determined using the following formulas (Eq.1-Eq.3) and expressed as mg 100 g^{-1} DW.

$$Chlorophyll\ a = 0.999 \times A^{663} - 0.0989 \times A^{645} \quad \text{(Eq.1)}$$

$$Chlorophyll\ b = -0.328 \times A^{663} + 1.77 \times A^{645} \quad \text{(Eq.2)}$$

$$\beta - carotene = 0.216 \times A^{663} - 1.220 \times A^{645} - 0.304 \times A^{505} + 0.452 \times A^{453} \quad \text{(Eq.3)}$$

2.4. Total phenolic compounds (TPC) content

The content of total phenolic compounds was determined using the procedures reported by Barros et al. (2011) as follows: 0.5 mL of each purslane extract were thoroughly vortexed with Folin-Ciocalteu reagent (0.5 mL) and Na_2CO_3 solution (1.5 mL, 20%). After adjusting the final volume to 10 mL the tubes were let to stand for 30 min at ambient temperature under dark conditions. Thereafter the absorbance was read at 760 nm against blank. A standard curve was created using gallic acid and the obtained regression equation ($y = 0.0984x - 0.1909$, $R^2 = 0.9941$) was employed to calculate the total phenolic content in the extracts as mg GAE (gallic acid equivalent) g^{-1} DW.

2.5. Total flavonoids content

The extracts' content of total flavonoids was measured spectrophotometrically according to the method reported by Pękal and Pyrzynska (2014) with slight changes. Briefly, purslane extract (0.5 mL) was combined with distilled water (2 mL) and $NaNO_2$ (0.15 mL, 5%, w v^{-1}). After 5 min, $AlCl_3$ (0.15 mL, 10%, w v^{-1}) was added to the mixture and the tubes were left at ambient temperature for 6 min. After neutralizing the mixture with NaOH (2 mL, 4%, w v^{-1}), the final volume was adjusted to 10 mL and the tubes were left for 10 min. The absorbance was measured at 510 nm against a blank. Rutin was used for creating the standard curve and the obtained regression equation (y= 0.0114x – 0.0079, R^2 = 0.9941) was employed to calculate the total flavonoids content in the extracts as mg RE (rutin equivalent) g^{-1} DW.

2.6. Total flavonols content

The extracts' content of total flavonols was tested according to the procedures described by Almaraz-Abarca et al. (2007) and Al-Dabbas (2017) with slight changes as follows: Purslane extract solution was vortexed with 0.5 mL of aluminum trichloride (0.5 mL, 2%, w v^{-1}), sodium acetate (0.5 mL, 5%, w v^{-1}) and distilled water (8.5 mL). Thereafter, the tubes were left at ambient temperature for 10 min and the absorbance was read at 425 nm against blank. A standard curve was created using rutin and the obtained regression equation (y = 0.0194x + 0.0038, R^2 = 0.9937) was employed to calculate the total flavonols content in the extracts as mg RE g^{-1} DW.

2.7. DPPH• scavenging activity

DPPH• scavenging activity of purslane extracts was determined following the method reported by Brand-Williams et al. (1995) with slight changes. Succinctly, a methanolic solution of DPPH (1.01 mM) was thoroughly mixed with purslane extracts at several concentrations (140 – 180 µL of extracts) and the final volume was brought to 4 mL using methanol. The absorbance was read at 517 nm at the end of the incubation period (30 min in dark at ambient temperature). The DPPH• scavenging activity (%) of each extract was assessed using equation 4 (Eq.4):

$$\text{DPPH} \bullet \text{ scavenging activity } (\%) = [(A^{\text{DPPH}} - A^{\text{sample}}) \times A^{DPPH^{-1}}] \times 100 \quad \text{(Eq.4)}$$

where A_{sample} refers to the absorbance of DPPH methanolic solution mixed with the sample, and A_{DPPH} refers to the DPPH methanolic solution without sample. BHA and α-tocopherol were used as control. The concentration (IC_{50}) of the extract causing scavenging of 50% of DPPH• was reckoned from the graph of extract concentration versus scavenging activity (%), and the IC_{50} value was expressed as µg mL^{-1}.

2.8. Ferrous iron (Fe^{+2}) chelating activity

The ferrous iron (Fe^{+2}) chelating activity was measured by mixing the purslane extracts (0.5 mL) with $FeCl_2$ (0.1 mL, 2 mM) and ferrozine solutions (0.2 mL, 5 mM). The tubes were then allowed to stand for 10 min at ambient temperature, thereafter, the absorbance was read at 562 nm against blank. The control was prepared by mixing $FeCl_2$ and ferrozine solution and the percentage ferrous iron (Fe^{+2}) chelating activity of various purslane extracts was assessed using the equation 5 (Eq.5) (Kumar et al., 2008):

$$\text{Ferrous iron } (Fe^{+2}) \text{ chelating activity } (\%) = [(A^{\text{control}} - A^{\text{sample}}) \times A^{control^{-1}}] \times 100 \quad \text{(Eq.5)}$$

2.9. Statistical analysis

Completely randomized-factorial design (2 purslane samples × 3 solvents) was used in this research. The results were expressed as a mean ± standard deviation of triplicates. To determine the differences between means, variance analysis followed by Duncan Multiple Comparison Test was conducted using the SPSS program (IBM SPSS Inc., version 20).

3. Results and Discussion

3.1. Fat-soluble pigments (β-carotene and chlorophylls a and b) contents of purslane extracts

Chlorophylls and carotenoids are natural pigments found in numerous edible plants. Chlorophyll a and chlorophyll b are the most widespread forms of chlorophylls and are usually found in higher plants at a ratio of 3 to 1, respectively. β-carotene (a vitamin A precursor) is one of the prominent and widespread members of carotenoids. However, many studies reported the association between these pigments and several health benefits including prevention and/or

protection against diverse types of cancer, coronary artery diseases and infections along with their roles as an antioxidant and anti-inflammatory agents (Mishra et al., 2011; Ghosh et al., 2018; Pérez-Gálvez et al., 2020). The content of β-carotene, chlorophyll a, and chlorophyll b in different purslane extracts were displayed in *Table 1*. As noted in the table, the acetone extracts exhibited the highest contents of β-carotene (14.16 mg g^{-1} DW), chlorophyll a (40.46 mg g^{-1} DW), and chlorophyll b (9.94 mg g^{-1} DW). Whereas the water extracts had remarkably lower amounts of these pigments, being 0.03, 0.03, and 0.06 mg g^{-1} DW, respectively. This could be explained by the polarity of the used solvent. Since chlorophylls and carotenoids are fat-soluble compounds, so they are expected to be more soluble in acetone (organic solvent) than methanol and water. By contrast, no significant differences between extracts obtained from the morphologically different purslane samples were observed. Several studies revealed purslane as a key source of various biologically active compounds particularly vitamin C, vitamin A, β-carotene, and α-tocopherol (Siriamornpun and Suttajit, 2010; Uddin et al., 2012a; Alam et al., 2014b; Youssef and Mokhtar, 2014).

Table 1. Fat-soluble pigments (β-carotene, chlorophyll a and chlorophyll b) contents (mg g^{-1} DW) of various extracts from purslane plant.

	Sample-1			Sample-2			Avg.		
	β-carotene	**Chlorophyll a**	**Chlorophyll b**	**β-carotene**	**Chlorophyll a**	**Chlorophyll b**	**β-carotene**	**Chlorophyll a**	**Chlorophyll b**
Water	0.01 ± 0.00^{Ab}	0.03 ± 0.00^{Ab}	0.06 ± 0.01^{Ab}	0.05 ± 0.00^{Ab}	0.03 ± 0.00^{Ab}	0.06 ± 0.00^{Ab}	0.03 ± 0.02^{c}	0.03 ± 0.00^{b}	0.06 ± 0.01^{b}
Methanol	3.06 ± 0.55^{Ab}	10.29 ± 1.25^{Ab}	1.26 ± 0.34^{Ab}	4.10 ± 0.06^{Ab}	12.45 ± 2.25^{Ab}	0.87 ± 0.17^{Ab}	3.58 ± 0.67^{b}	11.37 ± 2.01^{b}	1.07 ± 0.32^{b}
Acetone	12.15 ± 5.57^{Aa}	40.55 ± 22.94^{Aa}	13.22 ± 9.46^{Aa}	16.18 ± 2.89^{Aa}	40.36 ± 7.02^{Aa}	6.66 ± 0.99^{Aa}	14.16 ± 4.54^{a}	40.46 ± 15.17^{a}	9.94 ± 7.01^{a}

SD: standard deviation; a-c: means with different (small) letters within the same column are significantly different ($p < 0.05$); A-B: means with different (capital) letters within the same line and same compound group are significantly different ($p < 0.05$). Results were presented on dry weight basis.

3.2. Total phenolic, flavonoids, and flavonols contents of purslane extracts

Phenolic substances are secondary metabolites synthesized by plants and play critical roles in various functions at plants and are associated with numerous benefits for human health (Kumar and Pandey, 2013; Ávila-Román et al., 2021;). Purslane and its extracts are well known for their abundance of polyphenolics and strong antioxidant properties (Alam et al., 2014b; Hussien, 2016). The total phenolic, flavonoids, and flavonols contents of purslane extracts were showed in *Table 2*. Regarding the total phenolic content, purslane extracts varied significantly ($p < 0.01$) depending on the polarity of the used solvent. The highest amount of total phenolic compounds was detected in the acetone extracts (51.01 mg GAE g^{-1} DW) followed by methanol (45.90 mg GAE g^{-1} DW) and water (34.24 mg GAE g^{-1} DW) extracts in decreasing order. The solubility of phenolic substances is highly associated with their chemical structures, nature, and polymerization degree. In addition, phenolics are willing to interact with other plant components, including carbohydrates and proteins resulting in the formation of complexes with different solubility features. Moreover, the polarity of the solvent used in the extraction also influences the solubility and consequently the extractability of phenolic substances. Therefore, different amounts of various kinds of phenolic substances could be obtained from the same plant using different solvent systems (Naczk and Shahidi, 2006). The efficiency of acetone to extract higher levels of phenolic substances could be attributed to its capability to suppress the formation of protein-polyphenol complex throughout the extraction process or break down hydrogen bonds that formed between the carboxyl group of protein and phenolic group (Wang et al., 2009). Our findings were in accordance with those observed by Güngören (2016) who reported a higher amount of total phenolic compounds in purslane acetone extract compared to methanolic and aqueous extracts. Likewise, our findings were aligned with Uddin et al. (2012a) and Sallam et al. (2017) who reported higher values of total phenolic contents in purslane methanolic extracts than those in aqueous extracts.

The flavonoids content significantly varied from 7.65 mg RE g^{-1} DW in water extract of Sample-1 to 167.19 mg RE g^{-1} DW in acetone extract of Sample-1. In parallel with TPC results, acetone was also found to be more efficient to extract flavonoids from purslane samples with content of 133.23 mg RE g^{-1} DW followed by methanol and water being 99.47 and 10.98 mg RE g^{-1} DW, respectively (*Table 2*). On the other perspective, extracts obtained from two

morphologically different samples were found to be significantly ($p < 0.01$) different in terms of flavonoids content. Similarly, Uddin et al. (2012a) and Sallam et al. (2017) stated that methanol was more effective for flavonoid extraction compared to other solvents (ethanol and water). Regarding the flavonoids levels, our results showed that methanolic extract had higher flavonoids levels than what was reported previously by Uddin et al. (2012a) and Alam et al. (2014a) who reported content of 0.13 – 1.44 and 28.7 – 49.18 mg RE g^{-1}, respectively. The high values observed in the current study could be attributed to variations in various factors such as subspecies, varieties, growing conditions and extraction process.

Table 2. Total phenolic (mg GAE g^{-1} DW), flavonoids (mg RE g^{-1} DW) and flavonols (mg RE g^{-1} DW) contents of various extracts from purslane (mean ± SD).

	Sample-1			Sample-2			Avg.		
	Phenolics	**flavonoids**	**Flavonols**	**Phenolics**	**flavonoids**	**Flavonols**	**Phenolics**	**flavonoids**	**Flavonols**
Water	29.18±0.47^{Bb}	7.65±0.43^{Ac}	0.80±1.28^{Ac}	39.31±4.42^{Ab}	14.30±0.75^{Ac}	5.76 ±1.16^{Ab}	34.24±6.22^{c}	10.98±3.68^{c}	3.28±2.93^{c}
Methanol	47.03±3.57^{Aa}	128.18±12.18^{Ab}	18.41±5.63^{Bb}	44.78±5.03^{Ab}	70.76±3.06^{Bb}	46.19±4.03^{Aa}	45.90±4.09^{b}	99.47±32.44^{b}	32.30±15.84^{b}
Acetone	48.29±1.81^{Aa}	167.19±19.81^{Aa}	42.51±10.20^{Aa}	53.72±0.79^{Aa}	99.27±11.85^{Ba}	51.36±8.23^{Aa}	51.01±3.23^{a}	133.23±39.96^{a}	46.94±9.60^{a}

SD: standard deviation; a-c: means with different (small) letters within the same column are significantly different ($p < 0.05$); A-B: means with different (capital) letters within the same line and same compound group are significantly different ($p < 0.05$). Results were presented on dry weight basis.

In terms of flavonols content, a significant ($p < 0.01$) difference was spotted between extracts obtained from two morphologically different purslane samples (*Table 2*). The acetone extract had the highest flavonols content among the studied extracts, at 46.94 mg RE g^{-1}, succeeded by methanol (32.30 mg RE g^{-1}) and water (3.28 mg RE g^{-1}) extracts. Furthermore, the flavonols content of the water extract was found to be remarkably lower than that of other extracts (*Table 2*). Thus, the most effective solvent for flavonols extraction from purslane was also found to be acetone. It is difficult to compare the obtained results with other works because none have been published concerning total flavonols content in purslane extracts. However, Binici et al. (2021) stated that the total flavonols amount in fresh purslane varied between 3.48–17.27 mg RE g^{-1}. In addition, several studies indicated the presence of various substances that belong to the flavonol group such as rutin, myricetin, quercetin, and campherol in several parts of purslane plants (Siriamornpun and Suttajit, 2010; Erkan, 2012; Hassan, 2014). Furthermore, rutin was reported as the dominant flavonoid in purslane leaves and the major flavonoid in stems and flowers is myricetin (Siriamornpun and Suttajit, 2010).

3.3. DPPH• scavenging activity of purslane extracts

DPPH• was used to estimate the capability of various purslane extracts to scavenge free radicals. DPPH• scavenging activities of water, methanol, and acetone extracts obtained from purslane samples were displayed in *Figure 1*. As seen in the figure the DPPH• scavenging activities of the purslane extracts were increased as the dose increased. The purslane extracts exhibited antioxidant activity varied between 3.74% (water extract of Sample-2) and 28.29% (acetone extract of Sample-2). However, the two morphologically different purslane samples almost showed similar antioxidant activities. Whereas different extracts significantly ($p < 0.01$) varied in their antioxidant activities from 3.74 to 25.35%. In general, the minimum antioxidant activity was observed in water extracts, while the other extracts (methanol and acetone) revealed higher antioxidant activities. Furthermore, these two extracts (methanol and acetone) almost showed similar antioxidant activities with no significant ($p > 0.05$) differences between them. Similarly, Güngören et al. (2017) reported a higher DPPH• scavenging activity of the acetone extract (3.28 – 16.1%) obtained from different purslane samples compared to aqueous extract (1.64 – 16.77%). Another study revealed a higher antioxidant activity of the purslane methanolic extract (32.70%) compared to the aqueous extract (20.20%) (Sallam et al., 2017). However, the high antioxidant activity of acetone and methanol extracts in our study could be explained by their high content of TPC, flavonoids, and flavonols. It was pointed out that solvent polarity is strongly impacting the extractability of various antioxidant substances and therefore the antioxidant capacity of the extracts (Wang et al., 2009).

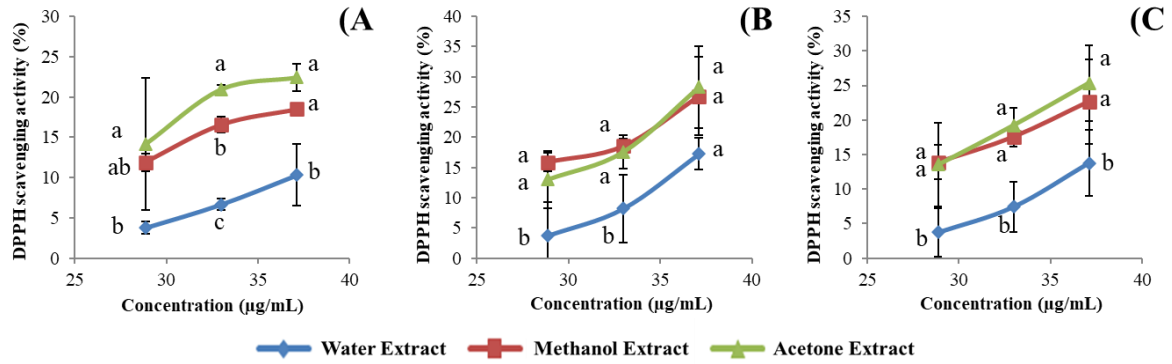

Figure 1. DPPH radical scavenging activity (%) of various extracts from purslane (A) Sample-1, B) Sample-2 and C) Average of both samples)

a-c: data points with different letters (within the same concentration) are significantly different ($p < 0.05$).

Regarding the IC_{50} value (half maximal inhibitory concentration) of purslane extracts, it was found to be between 50.65 µg mL^{-1} and 101.89 µg mL^{-1} (*Figure 2*). However, all extracts exhibited remarkably higher IC_{50} values than those of reference antioxidant substances (α-tocopherol: IC_{50} = 4.116 µg mL^{-1} and BHA: IC_{50} = 2.04 µg mL^{-1}). It is well known that the IC_{50} value is inversely proportional to the radical scavenging activity, i.e., a decrease in the IC_{50} value corresponds with an increase in radical scavenging activity. In the present study, the IC_{50} values were in parallel with the results of DPPH scavenging activity (%). Even though, no significant differences ($p > 0.05$) were identified between IC_{50} values of purslane extracts; in general acetone extract tends to have lower IC_{50} value compared to methanol and water extracts (*Figure 2*). Purslane is widely known as a rich source of various antioxidant compounds. The IC_{50} values of purslane methanolic extracts have been estimated to be between 2.50 – 3.29 mg mL^{-1} and 1.30 – 1.71 mg mL^{-1} according to Alam et al. (2014a) and Uddin et al. (2012a) respectively. Erkan (2012) reported the IC_{50} value of the methanolic crude extract of wild purslane as 511.8 µg mL^{-1}. In another study, the IC_{50} value of water extract obtained from purslane leaf was found to be 2.80 mg mL^{-1} (Siriamornpun and Suttajit, 2010). Our findings revealed stronger DPPH radical scavenging capacity with remarkably lower IC_{50} values than values obtained in the previous studies. However, several factors such as extraction technique, temperature, and time, as well as the size of the plant pieces and the used solvent (particularly solvent polarity) can affect the efficiency of the extraction process to extract certain groups of antioxidant substances and consequently the antioxidant features of the obtained extract (Naczk and Shahidi, 2006; Wang et al., 2009). According to the presented results, purslane extracts may consider as a promising antioxidant source.

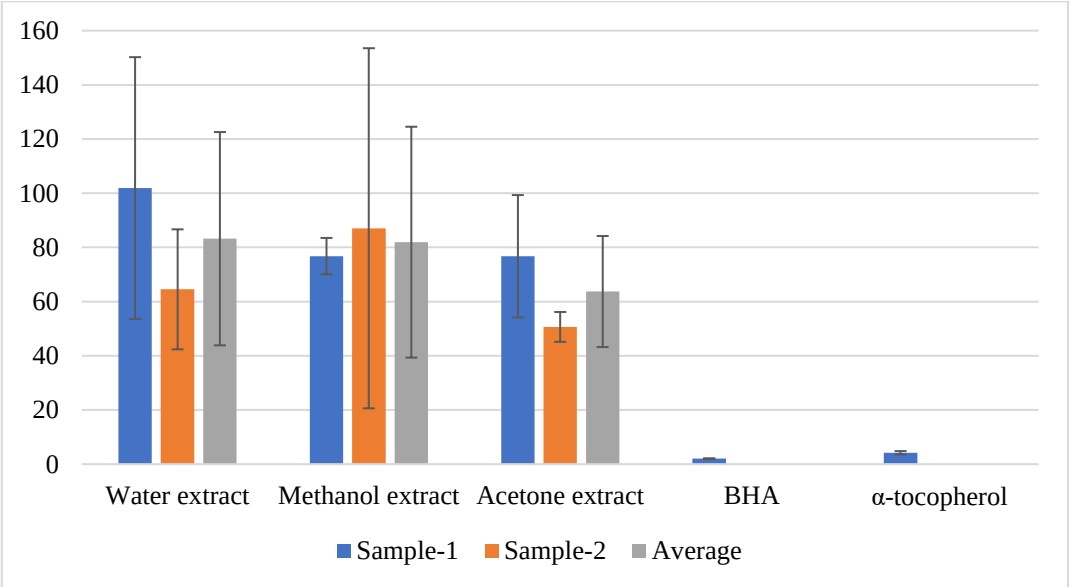

Figure 2. IC_{50} values of various extracts from purslane (µg ml^{-1}).

3.4. Ferrous iron (Fe^{+2}) chelating activity of purslane extracts

Transition metals such as iron, copper, and chromium have been suggested as catalysts in the formation of free radicals that can damage living cells. Substances that possess chelating activity serve as secondary antioxidants by binding metal ions and stabilizing them in the oxidized form, consequently inhibiting oxyradical formation and oxidative damage (Al-Dabbas, 2017). In the current study, the ability of various purslane extracts to capture ferrous iron (Fe^{+2}) ions was determined based on their capability to restrict the development of the ferrozine-Fe^{+2} complex. Obtained results revealed significant ($p < 0.01$) differences between extracts obtained from the two morphologically different purslane samples (*Table 3*). On the contrary, methanol extract showed higher metal chelating activity compared to the other extracts. Whereas acetone extract had the lowest chelating activity. Our findings were in accordance with Güngören et al. (2017) who stated higher ferrous iron (Fe^{+2}) chelating activity of aqueous purslane extracts (3.96 – 42.57%, at a concentration of 0.2 mg mL^{-1}) compared to acetone extracts (0.61 – 6.21%). Similarly, acetone extracts exhibited lower chelating activity than water extracts obtained from seaweeds according to Wang et al. (2009).

Table 3. Fe^{+2} chelating activity (%) of various extracts from purslane (mean±SD).

	Sample-1	**Sample-2**	**Avg.**
Water	5.82 ± 3.30^{Ba}	14.57 ± 3.05^{Aa}	10.20 ± 5.57^{b}
Methanol	8.69 ± 1.27^{Ba}	16.87 ± 1.15^{Aa}	12.78 ± 4.61^{a}
Acetone	1.50 ± 0.68^{Bb}	10.66 ± 0.64^{Ab}	6.08 ± 5.05^{c}

SD: standard deviation; a-c: means with different (small) letters within the same column are significantly different ($p < 0.05$); A-B: means with different (capital) letters within the same line are significantly different ($p < 0.05$). Extract concentration = 100 µg DW mL^{-1}.

As seen in *Table 3* various purslane extracts at a concentration of 100 µg mL^{-1} had ferrous iron (Fe^{+2}) chelating activity varied between 1.50 – 16.87%. Considering the extract concentration, values obtained in the current study were comparable to the values demonstrated by Güngören et al. (2017), contrarily it was remarkably higher than those stated by Peksel et al. (2006). The observed variation could be due to the differences in cultivars, growing conditions or extraction methods. It can be thought that the compounds showing metal chelating activity in plants were preserved better using our extraction method.

3.5. Correlation analysis

The correlation between the results of different assays is displayed in *Figure 3*. Correlation results revealed a strong positive relation between the extracts content of TPC with flavonoid (0.737, $p < 0.01$) and flavonol (0.756, $p < 0.01$) substances, proving the efficiency of acetone solvent in extracting polyphenolic compounds from purslane plant. Furthermore, TPC was also significantly correlated with β-carotene (0.708, $p < 0.01$) and chlorophyll a (0.664, $p < 0.01$) (*Figure 3*). On the other hand, DPPH• scavenging activity of purslane extracts was positively correlated with TPC (0.736, $p < 0.01$), flavonoid (0.823, $p < 0.01$) and flavonol (0.828, $p < 0.01$) compounds, indicating that polyphenolic substances could be the main contributors to the radical scavenging activity of purslane extracts. Moreover, a positive correlation was also observed between the scavenging activity of the extracts and their content of pigments including β-carotene (0.648, $p < 0.01$), chlorophyll a (0.678, $p < 0.01$), and chlorophyll b (0.544, $p < 0.05$). Several compounds including phenolics, flavonoids, flavonols, and various pigments are reported to exhibit a free radical scavenging activity (Miliauskas et al., 2004; Seyoum et al., 2006; Kumar and Pandey, 2013; Pérez-Gálvez et al., 2020;). In the purslane case, a good correlation between antioxidant activity including free radical scavenging activity with TPC and flavonoids contents was demonstrated by Youssef and Mokhtar (2014), Habibian et al. (2020) and Saffaryazdi et al. (2020), and A good correlation between DPPH• scavenging activity of purslane and its pigments content (chlorophyll a, chlorophyll b, carotenoids, and β-carotene) was also reported by Habibian et al. (2020) and Youssef and Mokhtar (2014). Regarding ferrous iron (Fe^{+2}) chelating activity, our findings showed that no significant ($p > 0.05$) correlation between chelating activity with β-carotene, chlorophyll a, TPC, flavonoids, flavonols, and DPPH scavenging activity. Wang et al. (2009) reported similar results in their study on seaweeds and suggested that the

chelating activity of the extracts was more effectively accomplished by other components such as polysaccharides, proteins, or peptides than phenolic compounds.

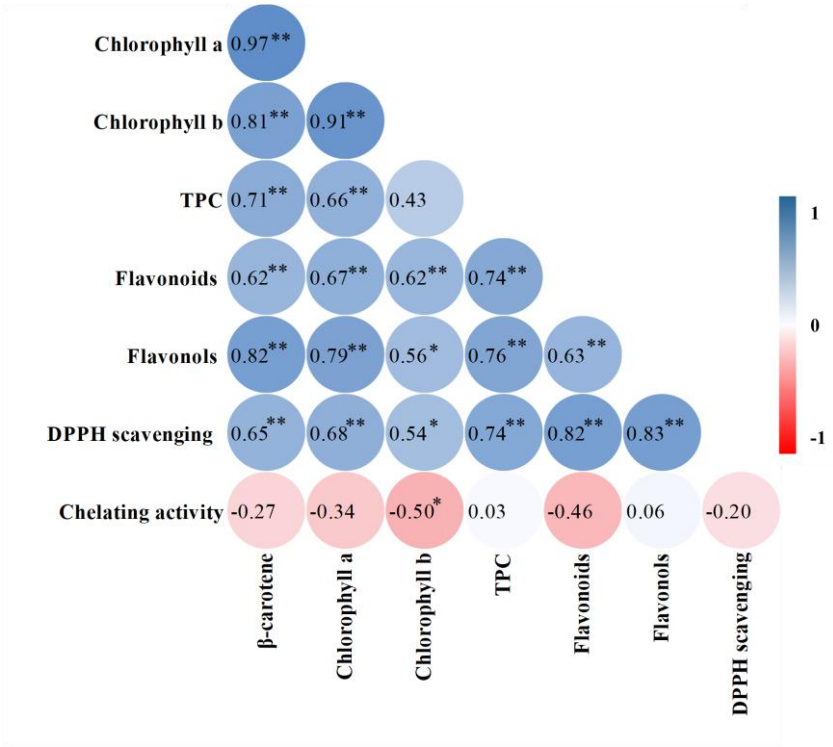

Figure 3. Pearson's correlation coefficient among different chemical attributes of purslane extracts. **: p < 0.01; *: p < 0.05

4. Conclusions

Investigating the chemical composition and antioxidant activity of various purslane extracts showed that the antioxidant activity was significantly affected by the solvent polarity, and the radical scavenging activity was mainly associated with the extract's content of phenolic compound. However, the acetone extract yielded the highest levels of the most studied parameters, while the lowest levels were found in the water extract. This is thought to be due to the capability of acetone to restrict the side interaction and/or the complexes formation throughout the extraction process. Unlike water and methanol, acetone has the advantage of evaporating quickly and easily at lower temperatures during the concentration of the extracts. In contrast, the acetone extract exhibited the lowest chelating activity, whereas the highest value was detected in the methanol extract. However, purslane extracts could be employed to naturally enhance the antioxidant properties of many pharmaceutical and food products.

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Ethical Statement

There is no need to obtain permission from the ethics committee for this study

Conflict of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Şat İ.; Supervision: Şat İ.; Methodology: Şat İ., Aoudeh E.; Formal analysis and investigation: Aoudeh E., Binici H.; Statistical Analyses: Aoudeh E., Binici H.; Writing - original draft preparation: Aoudeh E.; Writing - review and editing: Şat İ.; All the authors have read and agreed to the published version of the manuscript.

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