Study of the antioxidant potential of wild and cultivated Purslane (*Portulaca Oleracea* L.) available in Jordan

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Abstract

This study was conducted to measure the antioxidant capacity of the edible parts of wild and cultivated purslane varieties grown in Jordan. The samples were analyzed for total phenolic content using Folin-Ciocalteau method and total antioxidant capacity using ferric reducing antioxidant power (FRAP) assay. Purslane was also analyzed for vitamin C and oxalic acid contents. Results revealed that total phenolic contents of wild and cultivated purslane were 482.3 and 274.4 mg gallic acid equivalent (GAE)/100 g of dry plant (DP) respectively; the total antioxidant capacity values expressed by FRAP assay were 5.5 and 3.9 mM Fe^{+2} /100 g of DP, respectively. Vitamin C content was 35 and 30 mg/100g based on fresh weight, respectively. The oxalic acid content in wild and cultivated purslane was 2060 and 2990 mg /100 g of dry plant), respectively. In conclusion, purslane has a high antioxidant capacity with wild purslane having higher value than that of cultivated purslane.

Keywords: Purslane, Antioxidant capacity, Ferric Reducing Antioxidant Power Assay, Total phenolic content, Vitamin C, Oxalic acid

1. Introduction

Dietary antioxidant components are important for prevention of various degenerative diseases. The term antioxidant refers to compounds that inhibit or delay the oxidation of molecules like lipids through the inhibition of the initiation or propagation of oxidative chain reactions (Chanwitheesuk *et al*., 2004). Among the powerful dietary antioxidants are the biologically active polyphenolic components such as flavonoids and phenolic acids (Skotti *et al*., 2014). Chemically, polyphenols are a large heterogeneous group of compounds characterized by hydroxylated phenyl moieties (Cardona *et al*., 2013). Recent studies have shown that polyphenols in plants are more effective antioxidants than vitamin C and vitamin E *in vitro* (Chanwitheesuk *et al*., 2004).

Dietary polyphenols are among the most important groups of natural antioxidants found in human diet such as fruits, vegetables, grains and tea. Epidemiological, clinical and nutritional studies strongly support the evidence that dietary phenolic compounds enhance human health by lowering risk and preventing the onset of degenerative diseases including cancers, cardiovascular diseases and metabolic disorders (Zhou *et al*., 2015; Scalbert *et al.,* 2005). Some of the health benefits of polyphenols may not require their absorption through the gut barrier. The highest local concentration of polyphenols is found in the gut lumen (Rechner *et al.,* 2002). It is proposed that they may have a direct impact on the gut mucosa and protect it against oxidative stress. Also, polyphenols interact

with nutrients in the gut lumen (Jakobek *et al.,* 2015). They form stable complexes with non-heme dietary iron and limit its absorption in the gut (Jakobek *et al*., 2015).

Wild edible plants are defined as plant species collected in the wild to be consumed as food or drink. Historically, these plants have been used as an integral part in human diet in most countries of the world (Reyes-García *et al.,* 2015). Many wild edible plants are rich in nutrients including vitamins and minerals; therefore, they complement staple foods towards a balanced diet (Dogan *et al.,* 2013). These plants are rich sources of bioactive phytochemicals. As well, many of them have been recognized to have beneficial health impacts; such as anti-inflammatory, antimicrobial, and hypolipidemic effects (Wojdyło *et al*.,2007). Therefore, wild edible plants are effective in reducing the risk of many diseases and can be considered as functional foods (Romojaro *et al*., 2013).

In Jordan and other countries, wild edible plants are consumed in different ways and for different cultural reasons (Tukan *et al*., 1998). Early work on plants and vegetation have documented 142 wild plant species in Jordan, classified among 84 genera and 28 families (Al-Eiswi and Takruri, 1989). Many studies on the nutritive value and polyphenol content of some of the wild edible plants in the area were conducted. However, information regarding antioxidant capacity and phenolic content of wild edible plants in Jordan and the surrounding region is rare and not sufficiently documented (Tawaha *et al*, 2007).

Purslane (*Portulaca oleracea* L.) is one of the wild edible plants that are commonly consumed in Jordan (Tukan *et al*., 1998).Two types of purslane are found in many countries: the wild cultivar, which grows where water is available, and the cultivated (agronomic) cultivar (Osma *et al*., 2014).The present study aimed at measuring the total phenolic content, the antioxidant activity, vitamin C and oxalic acid in the 2 Jordanian cultivars of purslane: the wild and the cultivated.

2. Materials and methods

2.1. Plant collection

The selected wild and cultivated purslane plants were collected during late spring (May, 2018) from Amman area. Fresh plants that are not withered or in the flowering stage were collected. The plants were cleaned and the edible parts (leaves, puds and 5 cm of the stem) were seperated and dried in shade under low tempreture (20ºC to 30ºC) (Shanker et al., 2015). Samples were ground, transferred to glass containers and stored in the refrigerator at 4° C till analysed.

2.2. Chemical analysis

2.2.1. Vitamin C

Vitamin C or ascorbic acid was determined based on procedure described by Matei et al.(2004). In this method, vitamin C or ascorbic acid was determined in 10g wet plant by grinding the sample using mortar and pestle and homogenizing it with the extraction solution (10 ml/g). The extraction solution consisted of 15g metaphosphoric acid and 40 ml acetic acid in 500 ml distilled water. Then the filterate was titrated with indophenol solution.
The result was expressed as mg/100g fresh plant and it was calculated by this equation:

$$\text{Ascorbic acid} = \frac{(\text{volume consumed by sample} - \text{volume consumed by blank}) * \text{total volume} * DF * 100}{\text{volume of sample used for titration}}$$

DF: is Number of miligrams of ascorbic acid reacted with 1 ml indophenol standard solution.

2.2.2. Oxalic acid

Oxalic acid was measured as described by Naik et al. (2014) as follows: A dry sample of the plant weighing 0.5 g was extracted using 50 ml 0.25 N HCl with boiling in hot water bath for 15 minutes. After cooling, 10 ml of this solution were taken, centrifuged and then 2 ml of unhydrous sodium acetate was added. The solution was kept overnight and in the next day only the precipitate was dissolved in 5 ml HCl , washed by a washing reagent (1:1 ratio of ditluted ammonia solution and acetone) and recentrifuged. Finally the precipitate was dried in oven, dissolved in

5 ml H_2SO_4 and titrated against 0.02 N $KMnO_4$ solution which was standarized against 0.02 N oxalic acid solution with $KMnO_4$ as an indicator. The result was expressed as mg oxalic acid per 100 g dry sample.

2.2.3. Preparation of methanolic extract of samples

The extract was obtained by stirring 10 g of the dried and powdered plant with 250 ml of pure methanol followed by micro-filtration into cleaned tightly closed dry glass bottle. Finally, the bottle was shaded and kept in refrigerator at 4℃for further analyses (Ouerghemmi et al., 2017).

2.2.4. Yield determination

The Methanolic extract yield was determined by oven drying of 20 ml of extract at 60 C° using glass Petri dish, and then the calculation of the yield was done by the following equation.
Yield % = (weight of petri dish with sample before drying – weight of petri dish with sample after drying) / plant weight * 100

2.2.5. Determination of total phenolic content (TPC)

Total phenolic contents of the methanolic extracts were determined by using the Folin reagant (Folin – Ciocalteau) assay which was described by Ainsworth and Gillespie, (2007). The assay depends on the transfer of electrons in alkaline medium from phenolic compounds to phosphomolybdic /phosphotungstic acid complexes. A 100 µl sample extract solution was added to 0.5 ml folin reagent, then 1.5 ml of 15% sodium carbonate solution was also added to the mixture, and the volume was completed up to 10 ml by distilled water. Then after 30 minutes the absorbance was taken spectroscopically at 765 nm against blank. The result was expressed as mg gallic acid equivalent per 100g sample.

2.2.6. Ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power (FRAP) test which is based on the reduction of ferric 2,4,6- tripyridyl-s-traizine complex (Fe3+-TPTZ) to its ferrous, colored form (Fe2+-TPTZ) in the presence of antioxidants were conducted on the two extracts according to the procedure of Sharma and Sahu (2016), as follows:A 50 µl sample extract solution was mixed with 2.5 ml FRAP reagent of (10 mM TPTZ in 40 mM HCL, 20 mM $FeCl_3$ and 0.3 M acetate buffer PH 3.6). After incubation for 5 minutes at 37 C°, the absorbance was taken spectroscopically at 593 nm against blank. The results were given as mM Fe (II) equivalent per 100g using $FeSO_4.6H_2O$.

2.3. Statistical analysis

Statistical analysis of the data was performed using Statistical Package for the Social Science (SPSS) version 24. Data was expressed as mean ± standard error of the mean (SEM). Independent sample t-test was used to assess the difference between wild and cultivated species. The level of significance (P) was ≤0.05.

3. Results and discussion

3.1. Results

3.1.1. Vitamin C

Vitamin C content in the fresh plant is expressed as mg/100g of fresh plant. The vitamin C content in purslane is shown in table (1). It is noticed that vitamin C content of wild purslane was significantly different from that of cultivated purslane ($P<0.05$).

Table (1): Vitamin C and oxalic acid contents in purslane

Plant type	Vitamin C (mg / 100 g fresh plant)	Oxalic acid (mg /100 g dry plant)
Wild	35.0 ± 0.6[b]	2060 ± 0.1[a]
Cultivated	30.0 ± 0.6[a]	2990 ± 0.0[b]

3.1.2. Oxalic acid

Oxalic acid content in the plant extract which is expressed as mg oxalic acid per 100 g of dry plant, is shown in table (1). The oxalic acid was significantly lower in the wild cultivar than the cultivated.

3.1.3. Methanolic extract yield of Purslane

The methanolic extract yield for purslane on dry matter basis is shown in table (2). The extraction yield for wild purslane was 16.25% while extraction yield for cultivated purslane was 18.14%.

Table (2): Yield, total phenolic content (TPC) and ferric reducing antioxidant power activity (FRAP) in the dried plants

Plant type	Yield (%)	TPC (mg GAE/100g DP)	FRAP (mM Fe^{+2} / 100 g DP)
Wild	16.3%	482.3 ± 28.0[b]	5.48 ± 0.2[b]
cultivated	18.1%	274.4 ± 14.6[a]	3.91 ± 0.1[a]

3.1.3. Total phenolic content (TPC)

Total phenolic content (TPC) for the plant extract is expressed as mg gallic acid equivalent (GAE) / 100g of dry plant. The total phenolic content in the dry plants is shown in table (2); there is a significant difference ($P<0.05$) between wild and cultivated purslane regarding TPC content.

3.1.4. The ferric reducing antioxidant power (FRAP)

FRAP value of the plant extracts is shown in table (2). The FRAP value is expressed as mM Fe^{+2} / 100 g of dry plant. It was found that there is a significant difference between wild and cultivated purslane regarding FRAP assay values ($P<0.05$).

3.2. Discussion

Purslane has been reported to be a source of ascorbic acid (Zhou *et al*., 2015) which is among the antioxidant compounds. The vitamin C content of wild purslane was found to be 35.0 mg/100 g of fresh plant, which is significantly higher than that of the cultivated purslane (30.0 mg/100 g). These results are close to those reported

by Pellet and Shadarevian, (2013) who reported that purslane vitamin C content is 30 mg per 100 gram fresh weight, but higher than the value reported by Gonnella *et al*, (2010) which was 210 mg per kg of purslane on the basis of fresh weight). The value given by Kamal-Eldin *et al*, (2014) was 27 mg per 100 grams on fresh weight basis. These different values maybe explained by many reasons including the variation of the parts of plants used in the analysisas well as the ratio of soluble to insoluble oxalates.The main daily sources of dietary oxalate are plants and plant products. Oxalatesare available in plant tissue as soluble and insoluble salts. Soluble oxalate includes sodium hydrogen oxalate, potassium hydrogen oxalate and free oxalic acid. Poorly soluble oxalate salts are magnesium salts. Insoluble salts are calcium oxalate (Honow and Hesse, 2002).

In this study, wild purslane had anamount of 2060 mg oxalic acid /100 g of the dried plant, while cultivated purslane had 2990 mg /100 g dry plant. These resultsare consistent with those of Poeydomenge *et al*. (2007) who reported that the oxalic acid content of purslane equals 2345 milligrams per 100 gram of dry plant. The authors concluded that soluble oxalate was 37% and insoluble oxalate 63% of the total oxalate in the plant. They also reported that boiling the leaves, stems and buds resulted in decrease in soluble oxalates from the plant tissue which resulted in an overall 27% reduction in total oxalate in the tissues. Pickling the whole plant resulted in a loss of soluble oxalates from the tissue by leaching into the vinegar, resulting in a reduction of total oxalate content of the pickled tissue by 16%. Another study was done in Iran by Asadi *et al*, (2012) who examined some Iranian cultivars in the southern part of the country and showed that oxalic acid ranged between 40 mg to 80 mg per 100-gram on the basis of fresh weight. This discrepancy could be due to many factors; Palaniswamy *et al*. (2004) reported that oxalic acid content varies according to the harvest stage and the type of fertilizers used. It should be noted that in our study we did not determine the soluble and insoluble contents of oxalate which may explain the difference in the values of oxalate in our study from other studies.

Phenolic compounds such as tocopherols, polyphenols, phenolic acids, and lignans are widely distributed in plants (Wang and Ballington, 2007) and are very important antioxidants. These compounds are part of phytochemicals and possess phenolic ring attached with hydroxyl group; such hydroxyl groups enable the polyphenols to neutralize free radicals and work as efficient antioxidants (Zhang and Taso, 2016). Besides that, polyphenol compounds lower cholesterol level in blood (Kamal-Eldin *et al*., 2011) which is of interest to us in the present study.

In this study, the level of TPC was evaluated by the Folin-Ciocalteau, while the antioxidant activity of the plant (wild and cultivated purslane) was determined by ferric reducing antioxidant power (FRAP). We used methanol as an extractor for both types of plant; so, the values of TPC are based on that extractor. Total phenolic content (TPC) for the plant extract was calculated from gallic acid standard curve and expressed as mg gallic acid equivalent (GAE) / 100g of dry plant. TPC for the wild type purslane significantly differed ($P<0.05$) from that of cultivated purslane, TPC for wild was 482.3 mg GAE/100g for DP whereas for cultivated purslane,it was 274.4 mg GAE/100g for DP.

These findings are consistent with the results of the study of Lim, (2007) who worked on methanolic extracts of six cultivars of *Portulaca oleracea* that were analyzed for their total phenol content (TPC) using the Folin–Ciocalteau method. The author concluded that the TPC of the cultivars of *P. oleracea* ranged from 127 ± 13 to 478 ± 45 mg GAE/100 g on dry weight basis. Similar results were obtained in another study which was conducted by Uddin *et al*, (2012) who found that (TPC) varied from 142.8 ± 8.7 mg to 360.3 ± 8.9 mg GAE/100 grams on dry weight basis. That was also supported by El Kashef *et al*, (2018) who studied Egyptian purslane using several solvent extracts. They found that the total phenol content varied from 174.5±8.5 to 348.5±7.9 mg GAE/100 g on the basis of dry weight.

Generally, these variations in total phenolic content depend on different extraction procedures which could affect the total phenolic content of herbs and plants (Mikaili *et al*., 2013). Other workers have indicated that there are variations in total phenolic content within the same species, among different species, and sometimes among the different parts of the same plant such as flowers, stems, leaves, roots (Cartea *et al*., 2010).

Some factors in nature also affect total polyphenol content in plants and herbs. These include different stresses like insects and pathogens, biotic stress as sunlight, temperature, irrigation conditions, soil fertility, storage and processing conditions, harvest stage and the method of extraction used to determine bioactive compounds (Pandey and Rizv, 2009). The method of drying of the food to be analyzed is also vital in finding phenolic contents and antioxidants activity. It was reported that in drying process the oxidative enzymes are activated, leading to some loss in phenolic compounds (Kamiloglu *et al*., 2016).

On the other hand, using freeze-drying method will increase the antioxidant capacity. It is the best drying method since it has the lower temperature and pressure during the drying process (Valades-Carmona *et al*., 2017). These authors reported that the freeze-drying temperature can positively affect the activity, the content and bioavailability of polyphenols in tissues.In this study the FRAP value for wild purslane (5.5 mM Fe^{+2}/ 100 g of DP) was higher than that for the cultivated purslane (3.9 mM Fe^{+2} / 100 g of DP). These results are within the range of values reported by Kamal-Eldin *et al*, (2012) which is 1.8 ± 0.1 to 4.3 ± 0.1 mg GAE/g for the same plant.

4. Conclusion

The results of this study confirm the importance of purslane as a source of the antioxidant compounds, polyphenols and ascorbic acid.The wild cultivar has superiority in antioxidant capacity over the cultivated cultivar. It recommended to use other methods for the determination of the antioxidant capacity to support the results of the present investigation.

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