Journal of Applied Botany and Food Quality 91, 39 - 46 (2018), DOI:10.5073/JABFQ.2018.091.006

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Portulaca oleracea L. (Purslane) extracts display antioxidant and hypoglycaemic effects

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(Submitted: November 3, 2017; Accepted: February 7, 2018)

Summary

Purslane (*Portulaca oleracea* L.) is a member of the family Portulacaceae. Due to its many health benefits, it is listed in a World Health Organization database. The aim of this work is to investigate the purslane extracts for their chemical profile and bioactivity.

In this study, two different solvents (MeOH/H_2O and EtOH) were applied to fresh and dried leaves. The extracts were analysed using HPLC-DAD. Phenolic acids (caffeic acid, *p*-coumaric acid and ferulic acid) and flavonoids (apigenin, kaempferol, luteolin, quercetin, isorhamnetin, kaempferol-3-*O*-glucoside and rutin) were identified in all samples. Quercetin and *p*-coumaric acid were the most abundant compounds. Total antioxidant activity was measured by using the ABTS and DPPH tests, and the ferric reducing antioxidant power (FRAP) assay. Hypoglycaemic properties were investigated *via* the inhibition of carbohydrate-hydrolysing enzymes, α-amylase and α-glucosidase.

Fresh hydroalcoholic purslane extract exhibited the highest radical scavenging potential in both ABTS and DPPH test (IC_{50} values of 52.86 and 66.98 μg/mL, respectively), whereas dried hydroalcoholic purslane extract showed the highest α-glucosidase inhibitory potential (IC_{50} value of 45.05 μg/mL). Collectively these data show the health properties of this widely consumed salad plant.

Keywords: *Portulaca oleracea*, Phenols, HPLC-DAD, Antioxidant potential, Carbohydrate hydrolysing enzymes.

Introduction

Purslane (*Portulaca oleracea*) is an annual succulent plant of the family Portulacaceae. Originally, from India, today it has spread throughout the world's temperate zones, including Italy. The plant, which can grow to a height of 35 cm, has reddish-brown, prostrate, fleshy, branching stems, and light-green, fleshy, oval leaves. Although often considered a weed, all the aerial parts can be eaten, especially as a salad plant.

Purslane is rich in vitamins (Uddin et al., 2014), protein, carbohydrates and minerals (Uddin et al., 2014; Mohamed and Hussein, 1994) such as calcium, iron, magnesium, potassium, zinc and sodium (Aberoumand, 2009). Apart from is alimentary use, purslane has been traditionally used as a medicinal plant. It has anti-inflammatory and analgesic properties (Lee et al., 2012; Zhou et al., 2015; Rafieian-Kopaei and Alesaeidi, 2016; Meng et al., 2016), anticancer activity (Zhou et al., 2015; Lee et al., 2014; Ahangarpour et al., 2016) and antioxidant activity (Lim and Quah, 2007; Siriamornpun and Suttajit, 2010; Erkan, 2012). This plant can be used externally for various skin complaints, such as eczema, ulcers and acne, and to give relief from insect bites. It can also be used for coughs, bronchitis and fever (Zhang et al., 2002). Due to its many health benefits, it is listed in a World Health Organization database. Moreover, purslane is an excellent vegetable source of omega-3 fatty acids (Uddin et al., 2014; Liu et al., 2002; Simopoulos et al., 2005) since 100 g of purslane leaves contain around 350 mg of α-linolenic acid. Several works reported the presence of flavonoids as main bioactive purslane constituents (Erkan, 2012; Xu et al., 2006; Zhu et al., 2010).

In recent decades, the role of foods in disease prevention and treatment has been increasingly recognised. The role played by reactive oxygen species (ROS) in the pathogenesis of several diseases including diabetes mellitus (DM) has been clarified (Alfadda and Sallam, 2012; Tundis et al., 2016; Loizzo et al., 2017). To prevent complications, the stabilization of blood glucose levels in DM patients is crucial (Mai and Chuyen, 2007). Several therapeutic approaches may be used to achieve this objective: stimulating insulin release, increasing the amount of glucose transporters, inhibiting gluconeogenesis, reducing the absorption of glucose or decreasing the post-prandial hyperglycaemia (Kim et al., 2005). This last approach could be obtained by inhibiting carbohydrate hydrolysing enzymes α-amylase and α-glucosidase, using acarbose, voglibose and miglitol (Rios et al., 2005; Loizzo et al., 2016; Loizzo et al., 2017). However, these drugs are characterized by several gastrointestinal side effects including abdominal discomfort, flatulence, bloating, and diarrhoea. For these reasons natural sources are being investigated to provide new hypoglycaemic drugs (Rios et al., 2005; Tundis et al., 2010).

Both fresh and dried samples are used in medicinal plants studies. In most cases, dried samples are preferred considering the time needed for experimental design. Purslane is highly perishable in the fresh state; it has the shortest shelf life among fruits and vegetables due to its high metabolic reactions, which lead to loss quality. Therefore, the aim of this work is to investigate and compare the chemical composition, antioxidant and hypoglycaemic properties of dried and fresh purslane leaves.

Materials and methods

Chemicals and reagents

All reagents were of analytical grade and were purchased from Sigma-Aldrich S.p.a. (Milan, Italy). Acarbose from *Actinoplanes* spp. was obtained from Serva (Heidelberg, Germany). HPLC solvents were obtained from VWR International S.r.l. (Milan, Italy).

Sample, extraction and analysis procedure

Purslane plants were bought from a supermarket in Reggio Calabria (Italy) in November 2016. Leaves were manually separated from the stems and divided into two groups: fresh and dried (35 °C for 48 h). Samples were subsequently homogenized in a commercial blender and subjected to different extraction procedures: *a*) MeOH:H_2O (80:20 *v/v*) and *b*) 100% EtOH, in IKA Ultra-Turrax T25 and centrifuged for 10 min at 5000 rpm, after which the supernatant was filtered through a 0.45 mm Millipore filter (GMF Whatman) before analysis. For fresh leaves, yields (%) of 15.3 and 8.2 were obtained for hydroalcoholic and EtOH extracts, respectively, while for dried leaves, yields (%) of 10.7 and 6.6 were obtained for hydroalcoholic and EtOH extracts, respectively.

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Extraction of bioactive compounds and RP-HPLC/DAD analysis
RP-HPLC/DAD analyses of all samples were obtained as reported by Sicari et al. (2017) using a Knauer (Asi Advanced Scientific Instruments, Berlin) system equipped with two pumps Smartline Pump 1000, a Rheodyne injection valve (20 µL) and a photodiode array detector UV/VIS equipped with a semi micro-cell. Compounds were separated on a Knauer RP-C18 (250 mm × 4.6 mm, 5 µm). The chromatographic method used was a gradient elution of solvent A (water/formic acid, 99.9:0.1 v/v) and B (acetonitrile/formic acid 99.9:0.1 v/v). The gradient was used as follows: 0.01-20.00 min 5% B isocratic; 20.01-50.00 min, 5-40% B; 50.01-55.00 min, 40-95% B; 55.01-60.00 min 95% B isocratic. The column temperature was 30 °C and the flow rate was 1.0 mL/min. The injection volume was 20 µL. Peaks were monitored at 254, 330 and 305 nm. The identification and quantification of antioxidant compounds were carried out from the retention times in comparison with authentic standards. Data processing data were carried out using *Clarity* Software (Chromatography Station for Windows). All analyses were performed in triplicate and the results were expressed as mg/Kg of leaves.

Total phenolic content (TPC)
The total phenolic content of the extracts was determined as described by Singleton et al. (1999). An aliquot of 350 µL of extract was mixed with 1 mL of Folin-Ciocalteau reagent and 10 mL of 20% Na_2CO_3 solution. Absorbance was measured at $\lambda = 760$ nm using a UV-Vis Agilent 8453 spectrophotometer (Agilent Technologies, Italy) after 2 h in the dark. The results were expressed in milligram gallic acid equivalents per 100 g (mg/100 g) weight of the sample. All samples were analysed in triplicate.

DPPH Radical Scavenging Activity Assay
DPPH radical scavenging activity of *P. oleracea* was determined according to the technique previously described (Loizzo et al., 2016) at 517 nm using a UV-Vis Jenway 6003 Spectrophotometer.
The DPPH radical scavenging activity was calculated as follows: $[(A_0\text{-}A_1/A_0) \times 100]$, where A_0 is the absorbance of the control and A_1 is the absorbance in the presence of the sample. Ascorbic acid was used as positive control.

ABTS Radical Scavenging Activity Assay
As reported by Loizzo et al. (2016) the ABTS radical cation solution was mixed with potassium persulphate and left in the dark for 12 h. The ABTS solution was diluted with methanol to an absorbance of 0.70 ± 0.05 at 734 nm. After addition of sample (1-1000 mg/mL in methanol) to the ABTS solution, absorbance was measured after 6 min. Ascorbic acid was used as positive control.

Ferric Reducing Activity Power (FRAP) Assay
The FRAP assay is based on the redox reaction that involves TPTZ (2,4, 6-tripyridyl-*s*-triazine)-Fe^{3+} complex. FRAP reagent was prepared as previously described (Loizzo et al., 2015). Extracts were dissolved in methanol and tested at 2.5 mg/mL. BHT was used as control.

Carbohydrate hydrolysing enzymes inhibition study
A starch solution, α-amylase (EC 3.2.1.1) solution and colorimetric reagent were prepared. Both control and juice were added to starch solution and left to react with enzyme at room temperature for 5 min (Loizzo et al., 2014). The absorbance was read at 540 nm. The enzyme inhibition (%) was obtained by the following equation:

$$\%\ \text{Inhibition} = 100 - \left(\frac{[\text{Maltose}]\ \text{test}}{[\text{Maltose}]\ \text{control}} \times 100 \right) \pm \text{S.D.}$$

In the α-glucosidase inhibition test a maltose solution, α-glucosidase solution (EC 3.2.1.20) and *o*-dianisidine (DIAN) solution were prepared (Loizzo et al., 2014). A mixture of juice maltose solution and enzyme were left to incubate at 37 °C for 30 min. Then perchloric acid was added and mixture was centrifuge. The supernatant was collected and mixed with DIAN and PGO and left to incubate at 37 °C for 30 minutes. The absorbance was read at 500 nm. The α-glucosidase inhibition (%) was calculated by using the following equation:

$$\%\ \text{Inhibition} = 100 - \left(\frac{[\text{Glucose}]\ \text{test}}{[\text{Glucose}]\ \text{control}} \times 100 \right) \pm \text{S.D.}$$

Relative antioxidant capacity index (RACI) calculation
Relative antioxidant capacity index (RACI) is an integrated approach to evaluate the antioxidant capacity generated from different *in vitro* tests (Sun and Tanumihardjo, 2007). For the calculation, standard scores were used with no unit limitation and no variance among methods. Data obtained from TPC, ABTS, DPPH and FRAP tests were used to calculate RACI values for purslane samples.

Statistical analyses
Excel software (Office 2007) was used to calculate the means and the standard deviation. Statistical analysis was carried out using SPSS software for Windows (SPSS Inc., Elgin, IL, U.S.A.) 15.0 version. The means of all parameters were examined for significance using ANOVA with Tukey test to determine any significant difference between the treatments at $P < 0.05$. Further multivariate analysis was performed using Principal Component Analysis (PCA). All samples were analysed in triplicate.

Results and Discussion

Identification and quantification of the phenolic compounds present in *P. oleracea*
Both fresh and dried purslane leaves were analysed by HPLC-DAD to determine their bioactive compounds and the effect of drying on selected phenolic compounds. Tab. 1 reported the chemical profile of both fresh and dried purslane leaves extracts. Quercetin, apigenin, luteolin, kaempferol, isorhamnetin, kaempferol-3-*O*-glucoside and rutin were the flavonoids (Erkan, 2012; Zhu et al., 2012), and caffeic, *p*-coumaric, and ferulic acids were the phenolic acids found in all extracts (Siriamornpun and Suttajit, 2010; Erkan, 2012; Yang et al., 2009). Among identified flavonoids, quercetin was the most abundant compound with values in the range 16.01-6.02 mg/kg, followed by rutin (6.17-4.12 mg/kg) and kaempferol (3.25-1.85 mg/kg). Apigenin, luteolin, isorhamnetin, kaempferol-3-*O*-glucoside were found in smaller quantities (Tab. 1). The level of flavonoids depends on the portion of the plant, and is normally higher in the root, followed by the stem and leaves (Xu et al., 2006). *p*-Coumaric acid was the main phenolic acid with the highest value in hydroalcoholic fresh leaf extract (20.53 mg/kg) followed by EtOH dried sample (18.77 mg/kg). The same trend was found for ferulic acid.
Wide investigations have shown that *p*-coumaric acid and quercetin exhibit various bioactivities, including antioxidant, anti-inflammatory and anti-cancer activities, in addition to mitigating atherosclerosis, oxidative cardiac damage, diabetes and many other biological actions (Lim, 2007; Dkhil et al., 2011; Pei et al., 2015). In addition, quercetin is a versatile antioxidant known to possess protective abili-

Tab. 1: Quantification (mg/kg) of selected phenols of purslane leaves extracts.

	MeOH:H_2O (80:20 v/v)		**EtOH**	
	Fresh leaves	Dried leaves	Fresh leaves	Dried leaves
Flavonoids				
Apigenin	0.29 ± 0.05^a	0.11 ± 0.03^c	0.17 ± 0.05^b	0.09 ± 0.01^c
Kaempferol	3.25 ± 0.15^a	2.03 ± 0.12^c	2.75 ± 0.15^b	1.85 ± 0.14^d
Luteolin	0.55 ± 0.02^a	0.03 ± 0.08^d	0.32 ± 0.02^b	0.23 ± 0.03^c
Quercetin	16.01 ± 0.33^a	6.02 ± 0.03^d	11.11 ± 0.33^c	14.14 ± 0.30^b
Isorhamnetin	0.36 ± 0.01^a	0.08 ± 0.03^c	0.27 ± 0.01^b	0.25 ± 0.07^b
Kaempferol-3-*O*-glucoside	0.63 ± 0.05^a	0.23 ± 0.08^c	0.55 ± 0.01^b	0.53 ± 0.08^b
Rutin	6.10 ± 0.12^b	4.12 ± 0.04^d	5.11 ± 0.10^c	6.17 ± 0.12^a
Phenolic acids				
Caffeic acid	7.35 ± 0.08^a	3.48 ± 0.08^d	6.33 ± 0.24^b	5.58 ± 0.88^c
p-Coumaric acid	20.53 ± 0.46^a	11.03 ± 0.15^d	16.44 ± 1.18^c	18.77 ± 1.2^b
Ferulic acid	9.62 ± 0.41^a	4.12 ± 0.28^d	7.53 ± 0.88^c	9.27 ± 1.01^b
Sign.	**	**	**	**

Values are mean ± SD of three sample seed oils, analyzed in triplicate. Different letters indicate significant differences. Differences were evaluated by one-way analysis of variance (ANOVA) test completed with a multicomparison Tukey's test. **P<0.01 compared with the positive control.

ties against tissue injury induced by various drug toxicities (Anand David, 2016).
The efficiency of extraction varies according to the polarity of the solvent, pH, temperature, extraction time, and composition of the sample. When extraction time and temperature are the same, the solvent used and the composition of sample were shown to be the most important parameters. In the present study, extraction was performed on samples of purslane leaves using two different solvents: methanol/water (80/20 v/v) and ethanol (100%). Ethanol is known as a good solvent to extract phenol, as well as being safe for human consumption. Methanol is considered to be more efficient when extracting phenols of lower molecular weight (Dai and Mumper, 2010).
Huang et al. (2007) identified isorhamnetin, quercetin and kaempferol with values of 2.8, 1.3 and 1.1 mg/100 g, respectively. More recently, apigenin, bergapten, caffeic acid, *p*-coumaric acid, ferulic acid, scopoletin, quercetin, and quercetin-3-*O*-rhamnoside, were quantified in *P. oleracea* from different localities (Ai et al., 2015). Xu et al. (2006) identified mirycetin and luteolin.

Antioxidant potential

In this study, two *in vitro* tests, DPPH and ABTS, were used to screen the radical scavenging activity of *P. oleracea* extract. The total phenolic content and ferric reducing power was also tested. The different methods for measuring the radical scavenging potential can give different results according to which specific free radical is being used as a reactant. DPPH is often used to test how far compounds can act as free radical scavengers or hydrogen donors, and to quantify antioxidants in complex systems. The procedure which inhibits the production of the ABTS radical cation did not involve a substrate (Antolovich et al., 2002). The ferric thiocyanate method determines the quantity of peroxide, the main product of lipid oxidation, which is produced in the initial stages of oxidation. In this test, hydroperoxides formed from linoleic acid added to the reaction mixture, oxidized in air during the experiment, were indirectly measured. Antioxidants may be reductants and inactivation of oxidants by reductants are redox reactions in which one reaction species is reduced when the other is oxidized (Apak et al., 2004). Total phenols were calculated and expressed as gallic acid/100 g of extract. The highest value was found in fresh leaves hydroalcoholic extract (565 mg/100 g) followed by fresh leaves ethanol extract (488.04 mg/100 g) (Tab. 2).
A concentration-dependent activity was observed for all tested purslane extracts. Fresh purslane leaf ethanolic extract exerted the greatest DPPH radical scavenging activity with IC_{50} value of 52.86 mg/mL. This extract was followed by fresh leaf hydroalcoholic extract (IC_{50} value of 53.92 mg/mL) (Tab. 2). A similar trend was also seen in $ABTS^{\cdot+}$ radical scavenging ability where the IC_{50} values of 66.98 and 72.60 mg/mL were found for F and F1 samples, respectively. Generally, a promising ferric reducing ability was observed. In particular, fresh leaf hydroalcoholic extract showed a FRAP value comparable to the positive control BHT (56.11 *vs* 63.2 mM Fe (II)/g).
The RACI calculation was used to integrate the antioxidant capacity generated from different in *vitro* methods of each samples. For this calculation, the mean of standard scores was used, taken from the raw data of the different antioxidant tests. Any differences between units and in variances in the raw data did not influence the RACI. Stepwise regression between RACI and different tests demonstrated the following: 1) each test was selected as a significant variable with no one applied method being removed, 2) each method had the same weight in building RACI, and 3) the regression was highly significant ($r = 1$, $p < 0.001$). Based on RACI the following order of antioxidant ability was found: dried leaves (EtOH) > dried leaves (MeOH:H_2O) > fresh leaves (MeOH:H_2O) (Fig. 1). This trend plainly showed that dried leaves, independently of which solvent was used for extraction, had the highest antioxidant potential.
Recently, Youssef et al. (2014) reported the radical scavenging potential of purslane leaves fresh and under different drying procedures (hot-air drying, microwave drying and freeze-drying). Fresh purslane leave extracts showed values of 53.23% for DPPH and 147.78 µmol Trolox for ABTS per 100 g dry weight. All the examined methods of drying significantly lowered the antioxidant capacity of the sample. Analysis of data demonstrated that among investigated dried sample, hot air dried and freeze-dried purslane leaves retained a better antioxidant capacity independently by the temperature applied while microwave procedure drastically reduced the antioxidant potential of

Tab. 2: Total phenols content, radical scavenging activity and ferric reducing potential of purslane leaves extracts.

	Total Phenols (mg GAE/100 g)	DPPH (IC_{50} mg/mL)	ABTS (IC_{50} mg/mL)	FRAP (mM Fe(II)/g)
MeOH:H₂O (80:20 v/v)				
Fresh leaves	565.07 ± 3.23	52.86 ± 0.8	66.98 ± 1.9	54.35 ± 0.5
Dried leaves	244.17 ± 4.04	53.92 ± 1.3	72.60 ± 1.7	56.11 ± 3.9
EtOH				
Fresh leaves	488.04 ± 1.54	55.92 ± 1.1	85.91 ± 1.9	45.14 ± 3.3
Dried leaves	260.19 ± 2.07	56.87 ± 1.3	89.46 ± 2.3	36.22 ± 3.2
Positive control				
Ascorbic acid		2.0 ± 0.9	1.7 ± 0.8	
BHT				63.2 ± 2.8

Data are expressed as means ± S.D. (n = 3). DPPH Radical Scavenging Activity Assay: One-way ANOVA ***p<0.0001 followed by a multicomparison Dunnett's test: ***p<0.01 compared with ascorbic acid. Antioxidant Capacity Determined by Radical Cation ($ABTS^+$): One-way ANOVA ***p<0.0001 followed by a multicomparison Dunnett's test ***p<0.01, *p<0.05 compared with ascorbic acid. Ferric Reducing Antioxidant Power (FRAP): One-way ANOVA ***p<0.0001 followed by a multicomparison Dunnett's test **p<0.01 compared with positive control.

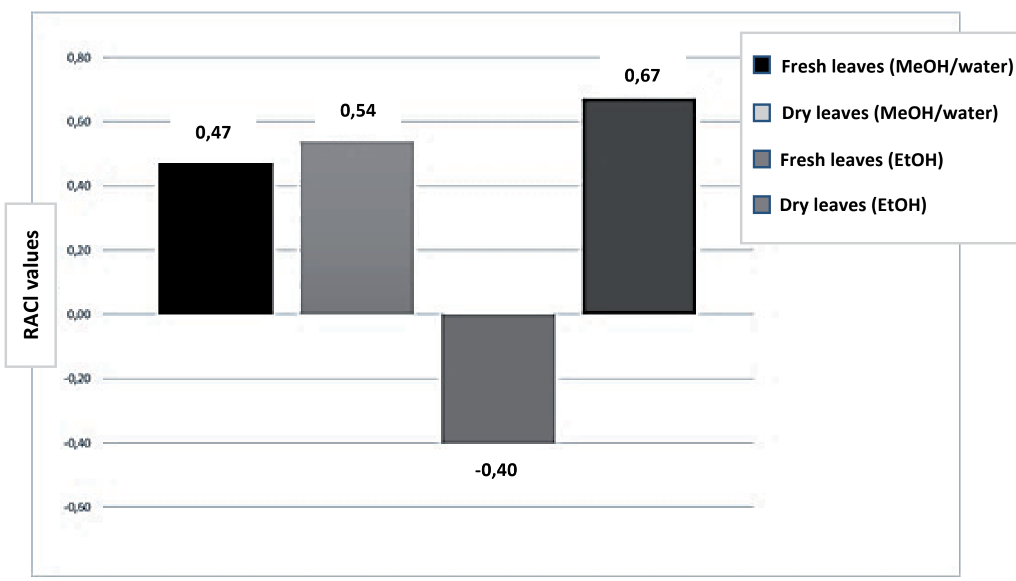

Fig. 1: Relative antioxidant capacity index of purslane leaves samples.

the matrix. These modifications in terms of bioactivity could be related to the different phytochemical content in investigated samples. However, the generation and accumulation of antioxidants during food dehydration may cause antagonistic or synergistic effects with each other or with other compounds present in the sample. The complex interactions influencing the functional properties of food during drying require further research (Hsu et al., 2003; Di Scala et al., 2011; Loizzo et al., 2013; Lòpez et al., 2013).

The methanol extracts edible fresh parts of thirteen *P. oleracea* from Malaysia were examined for their phytochemical content and antioxidant activity by using the DPPH radical scavenging method and FRAP assay (Alam et al., 2014). The IC_{50} values ranged from 2.52 to 3.29 mg/mL for DPPH test, and for 7.39 to 104.2 µmol TE/g DW for FRAP assay. These results are better than our data in terms of both radical scavenging activity and ferric reducing power. Differently, similar DPPH radical scavenging results were obtained with air-dried powered of Iranian *P. oleracea*. In fact, the *n*-hexane, dichloromethane, chloroform, ethyl acetate and methanol extracts showed IC_{50} values in the range from 62.9 to 91.0.8 mg/ml for ethyl acetate and dichloromethane extracts, respectively (Salehi et al., 2013). The influence of area of collection on the phytochemical content and antioxidant potential of this plant species was confirmed also by Silva et al. (2014) that investigated leaves, flowers and stems of *P. oleracea* from different area of Portugal. Results revealed that in the DPPH assay, samples from Vendas Novasr reached the 50% inhibition rate in lower concentrations than plants from Tavira. Recently, hydroalcoholic extracts of the aerial parts of *P. oleracea* from Bulgaria (POB) and Greece (POG) were studied for their radical scavenging activity and ferric reducing power (Gevrenova et al., 2016). Both purslane extracts revealed a similar radical scavenging potential with IC_{50} values of 1.98 and 2.00 mg/mL, and 0.88 and 0.92 mg/mL in DPPH and ABTS test for POB and POG, respectively. However, considering that all measured values are greater than those are found for the Calabrian extracts, our sample showed a most promising antioxidant potential. The sample from Bulgaria showed two-time highest ferric reducing ability respect POG with value of 0.16 mM Trolox equivalent.

Carbohydrate hydrolysing enzymes inhibition

Following our research interest in starch hydrolase inhibitors from edible plants we have investigate purslane extracts against α-amylase and α-glucosidase enzymes. These extracts inhibited carbohydrate-hydrolysing enzymes depending upon their concentration. Generally, α-glucosidase enzyme was most sensible since the IC50 values are in the range from 45.05 to 195.01 mg/mL for fresh leaf hydroalcoholic extract and fresh leaf ethanol extract, respectively. On α-amylase dried leaves, hydroalcoholic extract showed the highest inhibitory activity (IC_{50} value of 488.49 mg/mL) (Tab. 3). All these values are greater than those for the positive control acarbose.

Our values are in agreement with those reported by Salehi et al. (2013), which found an IC_{50} value of 93.2 µg/mL for *P. oleracea* methanol extract against α-glucosidase.

Previously, the effect of *P. oleracea* were screened *in vivo* in rats with type 2 DM. Results clearly evidenced that the extract reduced body weight, improved impaired glucose tolerance, attenuated hyperinsulinemia and elevated insulin sensitivity. The mechanism might be related to improved lipid metabolism and decreased free fatty acids (Lan and Fuer, 2003). Successively, El-Sayed (2011) studied the effect of *P. oleracea* seeds in thirty type-2 DM patients. Patients were split into two groups, one received 5 g of seeds two times a day, while the other received 1.5 mg of metformin daily. The treatment caused a significant drop in total cholesterol, low-density lipoprotein and serum levels of triglycerides and a rise in high-density lipoprotein. Other effects included modifications of liver transaminase, total and direct bilirubin, body weight and body mass index, fasting and post-prandial blood glucose, and insulin. In the metformin group similar effects were obtained.

Tab. 3: Carbohydrate hydrolysing enzymes of purslane extracts.

Assay	α-Amylase (IC_{50} mg/mL)	α-Glucosidase (IC_{50} mg/mL)
MeOH:H_2O (80:20 v/v)		
Fresh leaves	902.74 ± 3.8	195.01 ± 1.7
Dried leaves	640.01 ± 3.5	45.05 ± 1.1
EtOH		
Fresh leaves	774.02 ± 3.7	132.88 ± 2.1
Dried leaves	488.49 ± 3.5	138.51 ± 2.3
Positive control		
Acarbose	50.0 ± 0.9	35.5 ± 1.2

Data are expressed as means ± S.D. (n = 3). α-Amylase: One-way ANOVA ***p<0.0001 followed by a multicomparison Dunnett's test: ***p<0.01 compared with acarbose. α-Glucosidase: One-way ANOVA ***p<0.0001 followed by a multicomparison Dunnett's test: ***p<0.01 compared with acarbose.

Principal component analysis

PCA showed that the two principal components accounted for 95.12% of total variance, with PC1 for 61.24% and PC2 for 33.88% of total variance. The first principal component (Fig. 2) shows strong correlation with apigenin, kaempferol, luteolin quercetin isorhamnetin, kaempferol-3-*O*-glucoside, rutin, caffeic acid, *p*-coumaric acid, ferulic acid, total phenols, α-glucosidase and a lower correlation with α-amylase, DPPH, and ABTS test. This suggests that these thirteen variables are grouped together. In addition, from the analysis of variable loads, it was seen that the PC1 has a negative correlation with FRAP. The second principal component is strongly correlated with apigenin, kaempferol, FRAP and α-amylase, while it is strongly negatively correlated with DPPH and ABTS. Total phenols (TPC)

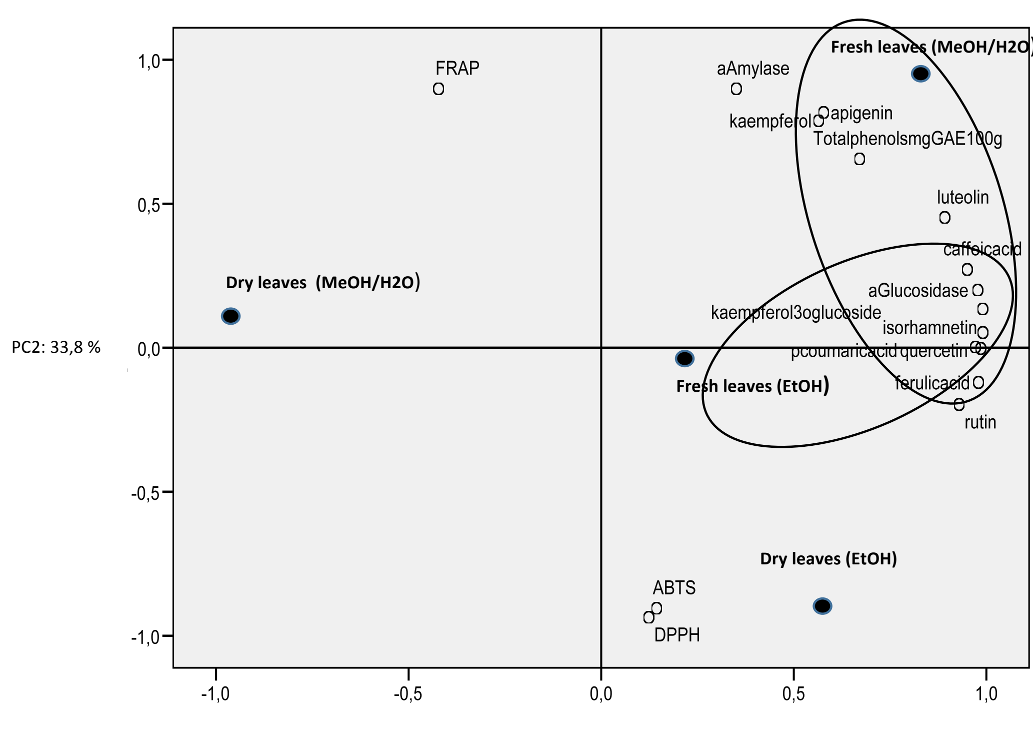

Fig. 2: PCA loading plot (PC1 vs PC2) for the first and second principal components.

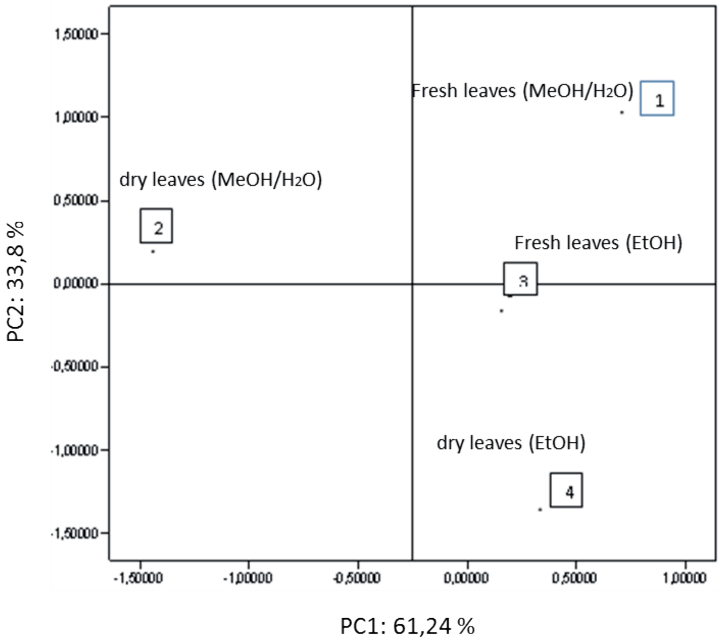

Fig. 3: PCA analysis of purslane extracts. Score plot of PC2 vs PC1.

is positively correlated with both PC1 and PC2. The significant correlations obtained support the hypothesis that phenolic compounds contribute significantly to the total antioxidant capacity (Cai et al., 2004; Djeridane et al., 2006; Sicari et al., 2016; Sicari et al., 2016a).

Fig. 3 shows that PC1 positive correlation with hydroalcoholic extracts obtained from the fresh leaves and was characterized by the presence of total phenols, flavonoid compounds, phenolic acids, α-amylase, α-glucoside and a low value of ABTS and DPPH. Moreover, values of the original variables are greater than those of the ethanolic extracts obtained from fresh and dried leaves, respectively. Methanol is the most suitable solvent in the extraction in polyphenolic compounds from plant tissue, due to its ability to inhibit the action of polyphenol oxidase that causes the oxidation of polyphenols (Yao et al., 2004; Lim and Quah, 2007). In addition, the different relationships between the antioxidant activity and the total phenolic content can be due to many factors; in fact the total phenolic content does not incorporate all the antioxidants. Also, it must be taken into account the synergism between the antioxidants in the extracts that makes the antioxidant activity not only dependent on the concentration, but also on the structure and the interaction between the antioxidants (Piluzza and Bullitta, 2011).

The ethanolic extract obtained from fresh leaves has a positive correlation with PC1, while the same obtained from the dried leaves shows low positive correlation and was characterized by assay of antioxidant activity (DPPH and ABTS). Hydroalcoholic extracts (dried leaves) were grouped at the negative side of PC1 (showing low flavonoid compounds, phenolic acids, total phenols and high FRAP values).

Conclusions

In this work, extracts from fresh and dried leaves of *P. oleracea* were tested to evaluate the most efficient process in terms of extracting bioactive molecules.

Was carried out a qualitative analysis of phenolic compounds present in the leaves of purslane examined by using LC-DAD by comparison with standard and literature data. Analysis of results revealed that fresh hydroalcoholic purslane extract exhibited a promising radical scavenging activity. A great difference was observed in hypoglycaemic whereas dried hydroalcoholic purslane extract exhibited the highest α-glucosidase inhibitory potential.

Therefore, the results of this paper, with the addition of further studies, will allow this plant with a large amount of biomolecules useful for beneficial effects on humans to be reevaluated. The richest extracts could be used in various fields such as the food and nutraceutical industries. In addition, due to its high content of nutrients, especially antioxidants, purslane is also a very likely candidate as a useful cosmetic ingredient.

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