## **Chemical composition and yield of six genotypes of common purslane (***Portulaca oleracea*

## **L.): An alternative source of omega-3 fatty acids**

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## **ABSTRACT**

Common purslane (*Portulaca oleracea* L.) is an annual weed rich in omega-3 fatty acids which is consumed for its edible leaves and stems. In the present study six different genotypes of common purslane (A-F) were evaluated for their nutritional value and chemical composition. Nutritional value and chemical composition depended on genotype. Oxalic acid content was the lowest for genotype D, whereas genotypes E and F are more promising for commercial cultivation, since they have low oxalic acid content. Genotype E had a very good antioxidant profile and a balanced composition of omega-3 and omega-6 fatty acids. Regarding yield, genotype A had the highest yield comparing to the other genotypes, whereas commercial varieties (E and F) did not differ from genotypes B and C. This study provides new information regarding common purslane bioactive compounds as affected by genotype and could be further implemented in food industry for products of high quality and increased added value.

## **Keywords**

Antioxidant activity· Bioactive compounds · Edible weed · · Oxalic acid ·Sugar content

# **Introduction**

Common purslane has been reported to contain several bioactive compounds [**1**]. Gharneh and Hassandokht [**2**] and Stroescu et al. [**3**] reported that common purslane is a rich source of omega-3 fatty acids and especially alpha-linolenic acid, which is considered beneficial against cardiac disorders [**4-6**]. Regarding its content in this specific fatty acid, purslane is considered one of the richest plant sources that can be used in human nutrition, having a high potential to be used as a functional food.

Oxalic and citric acids are the most abundant organic acids in purslane, whereas oxalic acid can combine with Ca and Fe and form oxalates which are insoluble and reduce Ca and Fe bioavailability and increase the incidence of kidney stone formation [**7-9**]. Purslane is also a rich source of dietary minerals such as K, Mg, P, Ca and Fe, whereas chemical composition is significantly altered during plant growth and dependent on planting date [**10-12**]. Naeem and Khan [**1**] report that purslane contains Ca and Mg in a ratio of 1:1. Moreover, purslane is a rich source of vitamin A and other antioxidants such vitamin C, tocopherols and beta-carotene [**10**]. According to the literature, the total phenolic content of purslane cultivars ranged from 127 to 478 mg GAE/100 g fresh weight and DPPH scavenging activity ranged from 2.52 to 3.29 mg/mL

[**13, 14**]. Furthermore, Oliveira et al. [**7**] reported that oxalic acid content in purslane showed a significant negative linear correlation with antioxidant activity.

Significant genetic variation exists among purslane genotypes for various agronomic traits [**15**], whereas the available genetic diversity could be a vast gene pool to be used in the development of high yielding cultivars with elevated omega-3 fatty acids content. Therefore, the objectives of the present study were: 1) the assessment of chemical composition of six purslane genotypes under Mediterranean environmental conditions, for future valorisation as novel food sources of omega-3 fatty acids, and 2) the evaluation of morphology traits and total yield of the studied genotypes

#### **Material and methods**

#### Study site

The experiment was carried out at the experimental field of University of Thessaly, at Velestino (central Greece) in 2014. The soil was loam (38% sand, 36% silt and 26% clay), the pH was 7.4 and the percent organic matter was 1.3 g/100 g soil. Temperature (mean, maximum and minimum air temperatures) and solar radiation (W  $m^{-2}$ ) throughout the growing season are presented in **Fig. 1** and **2.** The precipitation during the growing season (May-July) was 78 mm of rain. The soil was prepared according to the local practices for vegetable production, except that no chemical fertilizers were applied. Common purslane seeds were sown directly in soil on  $13<sup>th</sup>$  of May 2014, with distances between the rows at 60 cm, within the rows at 5 cm and sowing depth of 0.5 cm. Irrigation was applied via a sprinkler irrigation system immediately after sowing  $(13<sup>th</sup>$  of May) and two more times at weekly intervals (20 and  $27<sup>th</sup>$  of May). Weed control was applied by hand hoeing at regular intervals.

# Experimental Design

The experimental design was a completely randomized design (CRD) with four replications per treatment [six genotypes of common purslane, i.e. three wild ecotypes from Caspean sea region in Iran (36 °N, 53 °E) (genotype A: from Mazaderan Province, Sari city, genotype B: from Golestan Province, Gorgan city and genotype C: from Golestan Province, Aliabad city),one local population from "Domokos" region (D) in central Greece (39  $\degree$ N, 22  $\degree$ E) and two commercial cultivars (E: Common purslane from Gemma S.A. and F: Purslane Dark Green)]. Each experimental plot was  $16 \text{ m}^2 (4 \text{ x } 4 \text{ m})$ .

Sampling, measurements and methods

Harvest took place at 65 days after sowing (DAS) and plant height, internode length, stem diameter  $3<sup>rd</sup>$  node from the base), leaf length, width and thickness (4th pair of leaves from the apex), and leaf color (Chroma and hue angle) from 5 plants randomly selected from each plot was measured. For fresh and dry weight  $(kg ha<sup>-1</sup>)$  evaluation, an area of 1 m long corresponding to the central area of the middle two rows of each plot was hand harvested. The dry weight was determined after drying at 72 ºC until constant weight.

Color measurements were carried out with the implementation of Chroma Meter CR-400 (Konica Minolta Inc., Tokyo, Japan). Chroma values (C\* ) which according to McGuire [**16**] describe color saturation and hue angle (h<sup>o</sup>) which describes color shadiness ( $0^\circ$  = red-purple,  $90^\circ$  = yellow,  $180^\circ$  = bluish-green and  $270^\circ$  = blue), were determined according the following formulas:

$$
C^* = \sqrt{a^{*2} + b^{*2}},
$$
  
\n
$$
h^{\circ} = 180 + \left(\frac{(arctan\frac{b^*}{a^*})}{6.2832}\right) * 360, when a^* < 0 \text{ and}
$$
  
\n
$$
h^{\circ} = \left(\frac{(arctan\frac{b^*}{a^*})}{6.2832}\right) * 360, when a^* > 0.
$$

For chemical composition sampling, plant tissue samples (whole aerial parts) were taken from 15 plants from each genotype and all the samples were pulled in one and stored at deep freezing conditions (-80°C) and freeze dried prior to analysis. The freeze dried samples were powdered

with pestle and mortar and divided in three samples for further analysis. Organic acids were determined following a procedure previously described by the authors [**17**]. The analysis was performed using a Shimadzu 20A series UFLC (Shimadzu Cooperation, Kyoto, Japan). Free sugars were determined by high performance liquid chromatography coupled to a refraction index detector (HPLC-RI), after an extraction procedure previously described [**18**]. For the mineral composition, samples of plant tissues were dried in a forced-air oven at 72°C to constant weight, ground to powder, subjected to dry ashing and extracted with 1 N HCl to determine the minerals content. Ca, Mg, Fe, Mn, Zn, and Cu content were determined by atomic absorption spectrophotometry (Perkin Elmer 1100B, Waltham, MA) and Na and K content by flame photometry (Sherwood Model 410, Cambridge, UK).

For the antioxidant activity and bioactive compounds determination, the sample (1 g) was extracted twice by stirring (25 °C at 150 rpm) with 30 mL of methanol:water (80:20,  $v/v$ ) for 1 h and subsequently filtered through a Whatman No. 4 paper. The combined methanol:water extracts were evaporated at 40 ºC (rotary evaporator Büchi R-210, Flawil, Switzerland) to remove the methanol and further frozen and lyophilized. The extracts were redissolved in methanol:water (80:20, *v/v*) at a final concentration of 50 mg/mL and further diluted to different concentrations to be submitted to the distinct *in vitro* assays. The antioxidant activity of the methanol:water (80:20, *v/v*) extracts was evaluated by DPPH radical-scavenging activity, reducing power, inhibition of β-carotene bleaching in the presence of linoleic acid radicals and inhibition of lipid peroxidation using TBARS in brain homogenates [**18**].

Total phenolics were estimated by Folin-Ciocalteu colorimetric assay according to procedures previously described and the results were expressed as mg of Gallic acid equivalents (GAE) per g of sample [**19**]. Total flavonoids were determined by a colorimetric assay using aluminum trichloride, following procedures previously reported [**19**]; the results were expressed as mg of (+)-catechin equivalents (CE) per g of sample. Fatty acids were assessed after a transesterification procedure, according to Christie [**20**].

### Statistical analysis

For nutritional and chemical composition, three samples were analysed for each one of the purslane genotypes, whereas all of the assays were carried out in triplicate. The results were expressed as mean values and standard deviation (SD). The chemical composition and antioxidant activity were analysed using one-way analysis of variance followed by Tukey's HSD test with  $\alpha$ = 0.05 using the SAS v. 9.1.3 statistical program. Moreover, the sustainable yield index (SYI) was calculated as follows:

$$
SYI = \frac{Ym - Sd}{Y \max}
$$

were 'Ym' is the mean yield, 'Sd' the standard deviation and 'Ymax' is the maximum yield obtained under a set of management practices. A low value of SYI indicates unsustainable management practice [**21**].

### **Results and discussion**

Yield (fresh weight) was affected by genotype as clearly shown in **Figure 3**. The highest yield was found in genotype A  $(33182 \text{ kg ha}^{-1})$ . In addition, the lowest yield  $(11581 \text{ kg ha}^{-1})$  was recorded for genotype D whose growth was prostrate. The selected genotypes showed high sustainability yield index values. The SYI was greater for genotypes D and F (0.85 for genotype D and 0.89 for genotype F). The lowest SYI values were recorded for genotypes from Iran (A, B, C) and for commercial variety E (0.78, 0.71, 0.79 and 0.76 for genotypes A, B, C and E, respectively) (data not shown).

Significant differences in plant height were recorded, with all genotypes having upright growth, apart from genotype D whose growth was prostrate (**Table 1**). Moreover, genotypes A and E had the highest height (59.4 cm and 60 cm for A and E, respectively). For dry weight, no significant differences were observed between genotypes, whereas the lowest internode length was found in genotypes D, E and F and the lowest stem diameter was found in genotype E (**Table 1**). No significant differences in leaf length and width among the three genotypes from Iran (A, B, C) were observed, whereas the lowest leaf length, width and thickness were found in genotype E (**Table 2**). These results are in agreement with those of Egea-Gilabert et al. [**15**], who also observed significant differences between purslane accessions for all the morphological and agronomical traits (i.e. plant height, leaf area, internodes distance, number of total leaves).

Regarding colour parameters, the lowest Chroma value (26.55) was found in genotype E whereas no significant differences in hue angle (162.59-167.52) between the studied genotypes were observed (**Table 2**). In a previous study, it was reported that the hue angle in different purslane accessions ranged from 110.0 (accession CM 02-00297) to 115.5 (accession CM 02-00809), differences that could be attributed to different genotype and growing conditions [**15**].

Organic acids content in purslane is an important quality feature, especially for the oxalic acid which has a negative effect on human health and its low content in purslane foliage is considered a desirable trait [**22**]. The studied genotypes showed significant differences in total organic acids content, as well as in specific organic acids, namely oxalic, malic, citric and fumaric acid (**Table 3**). More specifically, oxalic acid content was the lowest for genotype D, which is an important quality feature for the commercial use of purslane. However, the fact that the specific genotype had the lowest yield has a counter effect and genotypes E and F seem to be more promising for commercial cultivation, since they have slightly higher oxalic acid content without trading off biomass yield (**Fig. 3a**).

In addition, it has been reported that the implementation of cultivation means such as increasing nitrogen fertilization with ammonium nitrogen and late harvesting, could alleviate the disadvantage of high oxalic acid content [**6, 8, 22**]. In our study, harvesting at 65 days after sowing (DAS) resulted in low oxalic acid content only in the case of genotypes A, B and E, whereas for the rest of the genotypes oxalic acid was significantly higher (**Table 3**). Therefore, genotype A which had the highest biomass yield could be commercially cultivated since the above mentioned cultivation means have been reported to significantly reduce oxalic acid content [**6, 8, 22**].

Significant differences were observed in total sugars content, where fructose, sucrose and glucose were the most abundant sugars for all the tested genotypes (**Table 3**). TSS content (°Brix) differed between the studied genotypes for both the stems and leaves, with genotype E having the highest <sup>°</sup>Brix in both stems and leaves (Fig. 3b). To our knowledge, this is the first time that sugars composition of purslane is assessed and could be an important quality feature that would define the organoleptic characteristics and taste and therefore consumers' acceptability of the final product.

Antioxidant activity of purslane is the key factor in order to propose its commercial cultivation as a health or functional food or as additive in food products intended for human nutrition. The studied genotypes showed significant differences in their antioxidants content (**Table 4**). Genotype E had the highest and second highest content in total phenolics and flavonoids, respectively, whereas its  $EC_{50}$  values were the lowest, indicating high antioxidant activity. Szalai et al. [**6**] have also reported significant differences in alpha and gamma-tocopherol content and total antioxidant activity of three purslane ecotypes, regardless of nitrate: ammonium nitrogen in nutrient solution.

Mineral composition of the studied genotypes differed significantly at the day of harvest (**Table 5**). Fe content was significantly higher for genotype D, whereas Ca did not differ between the genotypes A,B and C. Overall, the mineral content of the tested genotypes was higher than that reported by Uddin et al. [**10**], a difference that could be attributed to different genotype and growing conditions, since no further details were presented. In addition, our results are not comparable with the literature due to different expression of the mineral content [**11, 23**]. However, comparing to raw purslane composition reported from USDA [**24**], Ca and Mg content was higher for all the tested genotypes, whereas K and Zn was higher for all the genotypes except for genotype D. Fe content was similar with that reported from the USDA for the genotypes D and F, whereas the other genotypes had lower content (**Table 5**).

Purslane is considered a rich source of fatty acids, especially of omega-3 fatty acids such as alpha-linolenic acid which is the richest plant source reported so far [**5**]. Therefore its fatty acids content and composition could be considered a key quality factor for genotype evaluation. In our study, significant differences were observed among genotypes regarding their fatty acids content (**Table 6**). The most abundant fatty acids were palmitic (PA, C16:0), oleic (OA, C18:1) linoleic (LA, C18:2n6) and alpha-linolenic acid (ALA, C18:3n3), with significant differences among the genotypes. Genotype D had the highest relative percentage in alpha-linolenic acid. Oliveira et al. [**7**] have reported the same fatty acids to be the most abundant, however they detected significantly lower percentages for linoleic acid (4.00-6.31%) compared to our study (25.09- 32.90%). This difference may be attributed to the fact that they analysed the leaves separately from the stems, whereas in our study the results refer to whole plants (stems and leaves) since they are both edible, as well as to different genotype and growing conditions.

Polyunsaturated fatty acids (PUFA) accounted for the highest fraction of total fatty acids (FA) (48.58-56.12%). PUFA/SFA ratio was higher than 0.45 and ranged from 1.31 to 1.92, with great differences among the studied genotypes. Palmitic acid was detected at significant amounts (23.43-26.89%). , Moreover, linoleic acid was the prevailing fatty acid for all the genotypes with a ratio of omega-6: omega-3 ratio= 1.23-1.71, apart from genotype D where no significant differences between omega-6 and omega-3 fatty acids content were observed(omega-6: omega-3 ratio=0.88-1.02) .

Uddin et al. [**10**] have also reported that leaves of Thai wild purslane were rich in PUFAs and palmitic acid, however they detected significant amounts of gamma-linolenic acid (GLA, C18:3n6) which was not present in our study, a difference that could attributed to different genotype and growing conditions. In contrast, Oliveira et al. [**7**] reported higher percentages for SFAs than those for PUFAs for all the tested ecotypes, apart from one where PUFAs and SFAs were detected at similar amounts, whereas the omega-6: omega-3 ratio was significantly lower (0.14-0.28) compared to our study. These differences could be possibly due to genotype, the plant part and the harvest stage, since the samples were collected in situ without more available information about the growth stage of the plants when collected.

## **Conclusions**

Our results indicate that the tested genotypes had significant differences concerning chemical composition and nutritional value, especially regarding omega-3 fatty acids (alpha-linolenic acid) and oxalic acid content, which are considered the main quality features of purslane. The selection of the proper ecotype would be important means for food products of high quality and increased added value, in terms of high omega-3 fatty acids content, and low oxalic acid intake which has negative effects on human health. In conclusion, oxalic acid content was the lowest for genotype D, whereas genotypes E and F seem to be more promising for commercial cultivation, since they combine low oxalic acid content and high biomass yield. In addition, genotype E had a very good antioxidant profile and a balanced composition of omega-6 and omega-3 fatty acids.

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x=35&offset=&sort=&qlookup=purslane+raw (Accessed 2 September 2015). Table 1
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Influence of genotypes on plant height (cm), dry weight (g plant<sup>-1</sup>), internode length (cm) and stem diameter (mm) of common purslane

Genotype*	Plant height	Dry weight	Internode length	Stem diameter
$\mathbf{A}$	59.4a	1211a	5.67a	9.83a
B	53.8b	1797a	5.76a	10.65a
$\mathcal{C}$	51.4b	1744a	5.05b	9.66a
D	9.6d	1301a	4.46b	10.14a
E	60.0a	1638a	3.14c	6.21b
$\boldsymbol{F}$	44.2c	1713a	3.32c	10.22a
LSD <sub>5%</sub>	3.19	615	0.44	1.23

Means in the same column followed by different latin lower case letters are significantly different (P<0.05).\*A: Mazaderan Province, Sari city; B: Golestan Province, Gorgan city; C: Golestan Province, Aliabad city; D: Domokos, E: Common Purslane; F: Purslane Dark Green.

**Table 2** Influence of genotypes on leaf length (cm), leaf width (cm), leaf thickness (cm), colour and hue angle of common purslane

Genotype	Leaf length	Leaf width	Leaf thickness	Chroma	Hue angle
$\mathbf{A}$	4.15b	2.11ab	0.81b	33.04a	167.11a
B	4.31ab	2.00 <sub>b</sub>	0.87ab	33.21a	164.26 a
$\mathcal{C}$	4.55a	2.21a	0.82 <sub>b</sub>	34.76a	165.34 a
D	3.67c	2.00 <sub>b</sub>	0.93a	33.41a	167.52 a
E	2.22e	1.02d	0.71c	26.55b	166.07 a
F	3.24d	1.51c	0.87ab	34.05a	162.59a



Organic acids									
Samples	Oxalic acid	Malic acid	Citric acid	Fumaric acid	Total				
$\mathbf{A}$	$568 \pm 1b$	$23 \pm 1f$	$152 \pm 1b$	$1.8 + 0.1$	745±2b				
B	$524 \pm 2c$	$26 \pm 1e$	$88 \pm 1c$	$1.32 \pm 0.04d$	$639 \pm 2c$				
$\mathcal{C}$	$753 \pm 1a$	$104 \pm 1a$	$226 \pm 1a$	$2.5 \pm 0.1a$	$1086 \pm 1a$				
D	$371 \pm 1f$	$92\pm2b$	$151 \pm 1b$	$1.7 \pm 0.1c$	$616 \pm 1e$				
E	$452 \pm 1d$	$38 \pm 2d$	$48 \pm 1d$	$0.75 \pm 0.1$ f	$539 \pm 2f$				
$\mathbf{F}$	$432 \pm 1e$	$43 \pm 2c$	$151 \pm 1b$	$1.05 \pm 0.01e$	$627 \pm 1d$				
<b>Sugars</b>									
Samples	Fructose	Glucose	Sucrose	Trehalose	<b>Total Sugars</b>				
$\mathbf{A}$	$352 \pm 2a$	$59 \pm 2e$	$75 \pm 1f$	$42 \pm 2c$	$528 \pm 3d$				
B	$301 \pm 1c$	$81 \pm 1c$	$271 \pm 1a$	$133 \pm 3a$	785±5a				
$\mathcal{C}$	$305 \pm 1$	$118\pm1$ b	$175 \pm 3b$	$64\pm1$ b	$662\pm2b$				
D	$202 \pm 2e$	$67 \pm 1d$	$125 \pm 1d$	$26 \pm 1e$	$421 \pm 3e$				
E	$118 \pm 1f$	$52 \pm 1f$	$104 \pm 2e$	$19\pm 1f$	$293 \pm 3f$				
$\mathbf{F}$	$276 \pm 1d$	$138 \pm 1a$	$153 \pm 6c$	$35 \pm 1d$	$602 \pm 3c$				

**Table 3** Organic acids and sugars of the studied purslane genotypes (mg/100 g fw; mean ± SD)

Means in the same column followed by different latin lower case letters are significantly different (P<0.05). \*A: Mazaderan Province, Sari city; B: Golestan Province, Gorgan city; C: Golestan Province, Aliabad city; D: Domokos, E: Common Purslane; F: Purslane Dark Green.



**Table 4** Antioxidant properties of the studied purslane genotypes (mean  $\pm$  SD)

GAE- Gallic acid equivalents; CE- catechin equivalents. The results are presented in  $EC_{50}$  values, what means that higher values correspond to lower reducing power or antioxidant potential.  $EC_{50}$ : Extract concentration corresponding to 50% of antioxidant activity or 0.5 of absorbance for the reducing power assay. Means in the same column followed by different latin lower case letters are significantly different (P<0.05). \*A: Mazaderan Province, Sari city; B: Golestan Province, Gorgan city; C: Golestan Province, Aliabad city; D: Domokos, E: Common Purslane; F: Purslane Dark Green.



**Table 5** Mineral composition of the studied genotypes, expressed in mg/100 g of fresh weight (mean values ±SD)

Means in the same column followed by different latin lower case letters are significantly different (P<0.05).

\*A: Mazaderan Province, Sari city; B: Golestan Province, Gorgan city; C: Golestan Province, Aliabad city; D: Domokos, E: Common

Purslane; F: Purslane Dark Green.



**Table 6** Main fatty acids (relative percentage; mean  $\pm$  SD) of the studied purslane genotypes

Means in the same column followed by different latin lower case letters are significantly different (P<0.05). \*A: Mazaderan Province, Sari city; B: Golestan Province, Gorgan city; C: Golestan Province, Aliabad city; D: Domokos, E: Common Purslane; F: Purslane Dark Green.



**Figure 1** Temperature (max, min and mean values in °C) for the experimental site during the growing period (May-July 2014)







**Figure 3** Effect of genotype on: a) yield ( $kg$  ha<sup>-1</sup>) and b) total solids content ( $\langle Brix \rangle$  of common purslane. Means followed by the same latin lower case letter are not significantly different according to LSD test (p=0.05). \*A: Mazaderan Province, Sari city; B: Golestan Province, Gorgan city; C: Golestan Province, Aliabad city; D: Domokos, E: Common Purslane; F: Purslane Dark Green