Antioxidant activity and fatty acids quantification in Sicilian purslane germplasm

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\textit{Portulaca oleracea} is an annual succulent herb in the family Portulacaceae. It is a nutritious vegetable with high antioxidant properties and, it is among the richest plant source of n-3 fatty acids, as well as a rich source of n-6 fatty acids, ascorbic acid, tocopherols and beta-carotene. In the present study, three purslane populations under different Mediterranean environmental conditions for two years, for future valorization as novel food sources of omega-3 fatty acids, were evaluated. In particular, biomorphological characteristics, total phenols and fatty acids content were determined. The antioxidant activities were evaluated using 2,2-diphenyl-1-picrylhydrazyl assay. The population “Cas” appears to have higher antioxidant activity than the other two populations (“Cal” and “S. Ven”).The saturated fatty acid content is influenced only by the year of collection, while the polyunsaturated fatty acid by the populations. The most abundant unsaturated fatty acids are linoleic and linolenic acids and “Cas” attained the highest contents.
Supplemental file

Experimental section

Plant material and sites of collection

Three different populations of purslane, namely Caltagirone (Cal), Cassibile (Cas) and Santa Venerina (S. Ven), were harvested from native plants found in different areas of eastern Sicily, during the months of July 2016 and 2017. In the identified sites of collection the plant resulted widespread and cover naturally the degraded soils. The characteristics of the sites of collections are reported in table S1.

The aboveground biomass was harvested out in all sites of collection the July 2016 and 2017. In each site of collection twenty representative plants were harvested.

In laboratory harvested plants were immediately weighed in order to determine the fresh weight (fw). The moisture content of biomass components (stems, leaves and roots) was measured by weighing 100 g of plant material in a precalibrated porcelain capsule and placing it in a thermoventilated oven at 105 °C until constant weight was reached. On fresh leaves the color indices L *, a *, b * (Minolta colorimeter, CR 400) according to ICC-152 method (Cauvain & Young 2009) were measured. All analyses were performed in triplicate for each sampling and are reported on a dry matter (DM) basis.

Biomass production per plant was expressed as g plant\(^{-1}\) DM. Collected parts of the plant were blended thoroughly for homogeneity and washed with deionized water. After draining excess water, the biomass (leaves + stalks) was dried at 40 °C and ground into powder for further chemical characterization.

Chemical analyses

Total phenol content (TPC): TPC was determined using Folin-Ciocalteu method as reported by Singleton et al. (1998). The antioxidant activity was evaluated using the DPPH\(^{-}\) (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity (Brand-Williams et al. 1995).

FAMEs analysis was determined using GC/MS as reported by Pantano et al. (2016) and Cicero et al. (2018) with some modifications: 0.5-g amount of dried plant sample was mixed with 2 mL of CHCl\(_3\) and 1 ml of MeOH; the sample is sonicated for 45 minutes at 50 °C and then cooled to room temperature; centrifuged at 4000 rpm for 5 minutes.
and the supernatant was filtered with a 0.45 μ syringe filter and evaporated to dryness under a gentle stream of nitrogen. The residue was dissolved in 100 L of toluene and 200 μL of KOH in MeOH (10% w/v). The samples were vortex-for 5 min, and 200 μL of water and 1 ml of hexane were added. The upper layer containing FAMEs was collected and 500 μL were added to 100 μL of Internal standard solution (methyl ester Heneicosanoic acid 50 μg/mL) and 400 μL of hexane prior to GC/MS analysis. For the separation and analysis of the fatty acid methyl esters from Portulaca dried extracts, Thermo Scientific DSQ II single quadrupole system in EI (Electron Ionization) mode, working in full scan was used. A ZB-WAX (30 m x 0.25 mm i.d., 0.25 μm film thickness (Phenomenex, Italy)) was employed. The following chromatographic conditions were used: column oven temperature from 40 °C to 250 °C (10 min hold) at 2 °C/min; injector and detector temperatures 250 °C; Helium was used as the carrier gas at a flow rate of 0.8 mL/min. A sample of 1 μL was injected with a split ratio of 1:100.

Mass spectroscopy conditions: ionization voltage was 70 eV and the mass range scanned was 35-550 m/z. Using Thermo Scientific Xcalibur Data system software for Windows peak areas were determined and identified by comparison of retention times with those of a FAMEs standard mix (Supelco FAME Mix C8-C24) separated under the same chromatographic conditions. Triplicate analyses were prepared for each dried plant sample. FAMEs are expressed in mg g⁻¹.

**Data analysis**

Data were submitted to the Bartlett’s test for the homogeneity of variance and then analysed using analysis of variance (ANOVA). Means were statistically separated on the basis of Ducan’s test, when the ‘F’ test of ANOVA for treatment was significant at least at the 0.05 probability level. Significance was accepted at P < 0.05 level. The coefficient of variation (CV%) was calculated by using standard statistical procedures (Snedecor and Cochran 1989).

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### Table S1 Sites of collection and their characteristics.

| Location       | Coordinates          | Altitude (m a.s.l.) | Yearly average temperature (°C) | Yearly average Precipitation (mm) | De Martonne aridity Index (a) | Soil Characteristics (b) |
|----------------|----------------------|---------------------|----------------------------------|-----------------------------------|------------------------------|--------------------------|
| Caltagirone   | 37°11’07’’ N 14°13’19’’ W | 405                 | 15-18°C                          | 400-500 mm                        | Semi-arid                    | Haplic e Petric Calcisol; Calcic, Chromic e Skeletic Luvisol; Calcaric e Luvic Phaeozem; Calcaric Fluvisol; Haplic e Calcic Vertisol; Calcic Kastanozem; Eutric, Fluvic, Endogleyic e Calcaric Cambisol; Vitric Andosol; Calcaric Regosol; Calcaric Arenosol. |
| Cassibile     | 36°58’33’’ N 15°12’18’’ W | 48                  | 18-19°C                          | 500-600 mm                        | arid                         | Warm temperate climate    |
| Santa Venerina| 37°40’23’’ N 15°19’26’’ W | 201                 | 18-19°C                          | 800-1000 mm                       | humid                        | Humid temperate climate   |

(a) Drago A. 2005. Atlante Climatologico Della Sicilia – Seconda Edizione. Riv Ital di Agrometeorol. 83:67–83.
(b) Costantini EAC, L’Abate G. 2016. Beyond the concept of dominant soil: Preserving pedodiversity in upscaling soil map. Geoderma 271; 243-253 [Internet] Available from: https://doi.org/10.1016/j.geoderma.2015.11.024

47 - Haplic e Petric Calcisol; Calcic, Chromic e Skeletic Luvisol; Calcaric e Luvic Phaeozem; Calcaric Fluvisol; Haplic e Calcic Vertisol; Calcic Kastanozem; Eutric, Fluvic, Endogleyic e Calcaric Cambisol; Vitric Andosol; Calcaric Regosol; Calcaric Arenosol.

43- Calcic, Sodic, Gypsic e Haplic Vertisol; Fluvic and Calcaric Cambisol; Calcic Luvisol; Gypsic Regosol; Calcic e Haplic Gypsisol
40 - Leptic Luvisol; Luvic, Haplic e Calcaric Phaeozem; Calcaric Leptosol; Dystric Andic e Calcaric Cambisol
Table S2 Analyses of variance of the population characteristics and partitioning of the treatment sum squares (SS expressed in absolute value – AV – and percent of total) into main effects and interactions.

|                          | Population (P) | Year of collection (Y) | Interaction (P x Y) |
|--------------------------|----------------|------------------------|---------------------|
|                          | AV %           | AV %                   | AV %               |
| Stems (g plant⁻¹ d.m.)   | 28.58          | 69.62 **               | 0.47               | 1.15 n.s. 12.00 | 29.23 * |
| Leaves (g plant⁻¹ d.m.)  | 1.91           | 42.83 n.s.             | 1.63               | 36.60 n.s. 0.92 | 20.57 n.s. |
| Roots (g plant⁻¹ d.m.)   | 0.16           | 83.85 n.s.             | 0.01               | 2.60 n.s. 0.03 | 13.54 n.s. |
| Total biomass (g plant⁻¹ d.m.) | 51.32          | 73.30 **               | 4.65               | 6.64 n.s. 14.04 | 20.05 n.s. |
| Incidence of stems (% of total biomass) | 152.65          | 5.99 n.s.              | 857.95             | 33.67 *** 1537.77 | 60.34 *** |
| Incidence of leaves(% of total biomass) | 216.82          | 8.29 *                 | 1016.65             | 38.88 *** 1381.20 | 52.82 *** |
| Incidence of roots (% of total biomass) | 56.63          | 67.48 n.s.             | 7.59               | 9.05 n.s. 19.69 | 23.47 n.s. |
| L*                       | 320.24         | 60.78 ***             | 136.84             | 25.97 ** 69.84 | 13.25 n.s. |
| a*                       | 87.16          | 62.79 ***             | 34.89              | 25.14 ** 16.76 | 12.07 * |
| b*                       | 341.07         | 88.20 ***             | 6.82               | 1.76 n.s. 38.81 | 10.04 n.s. |
| TPC (mg 100⁻¹ D.W.)      | 1658           | 91.3 ***              | 21.3               | 1.2 n.s. 126 | 7.00 n.s. |
| IC₅₀ (mg/ml)             | 1.12           | 89.6 ***              | 0.11               | 8.8 *** 0.02 | 1.6 ** |
| Palmitic Acid (mg/g)     | 0.27           | 9.93 n.s.             | 2.22               | 80.31 *** 0.27 | 9.75 n.s. |
| Stearic Acid (mg/g)      | 0.01           | 1.04 n.s.             | 0.09               | 7.38 ** 1.06 | 91.58 n.s. |
| Oleic Acid (mg/g)        | 0.29           | 45.93 ***             | 0.24               | 37.96 *** 0.10 | 16.11 n.s. |
| Linoleic Acid (mg/g)     | 2.26           | 85.68 ***             | 0.04               | 1.41 n.s. 0.34 | 12.91 n.s. |
| Linolenic Acid (mg/g)    | 2.70           | 67.58 ***             | 0.03               | 0.83 n.s. 1.26 | 31.60 * |

*** Significant at 0.001 probability level
** Significant at 0.01 probability level
* Significant at 0.05 probability level
n.s. not significant