Article

Phenolic compounds and antiradical capacity of five wild accessions of *Portulaca oleracea* L. obtained with three solvents

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Abstract

Purslane (*Portulaca oleracea* L.) is a species recognized for its high levels of bioactive compounds, among which the compounds with antioxidant properties stand out. The objective of the work was to determine the variation of phenolic metabolites and their antiradical capabilities in wild accessions of *P. oleracea*. The research was conducted with samples from five municipalities in Tamaulipas, Mexico collected in October 2018. The contents of total phenolic compounds (CFT) and free radical scavenging capacity against ABTS (2,2'-azino-bis- (3-ethylbenzothiazoline-6-sulfonic) acid) and DPPH (2,2-diphenyl-1-picrylhydracyl) were determined. Extractions were made from vegetative parts using three solvents: acetone, water and ethanol. The accession of the localities of Abasolo and Padilla were the ones that had the highest amount of CFT with 5.8938 ±0.03 and 5.3742 ±0.11 mEAG g^{-1} PS, respectively, using water in the extraction. Regarding the free radical scavenging capacity against ABTS, the accession of Abasolo and Jiménez recorded the highest values, with 3.27 ±0.06 and 3.2226 ±0.04 mM ET g^{-1} PS. Regarding the level against DPPH radicals, the accession of Abasolo was the highest with 2.0204 ±0.05 mM ET g^{-1} PS, using water in the extraction. Water was the best solvent for the extraction of the determined contents. Heterogeneity was observed in the composition and levels of the parameters evaluated among the accessions. Wild *P. oleracea* accessions represent reservoirs of phenolic compounds and free radical scavenging capacity, including cultivated and wild varieties.

Keywords: antioxidants, edible plant, phytochemical variation.

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Introduction

Plant products with antioxidant contents are considered efficient to reduce oxidative stress, therefore, they are consumed to reduce this process in cells. The characterization of the antioxidant activity of edible plants is the basis for being able to relate them to potential biological functions, beneficial effects and for programs of improvement and use of crops (Top *et al*., 2014; Urbizu-González *et al*., 2017).

Phenolic compounds are considered antioxidants and have been reported in many species of fruits, vegetables and cereals consumed as part of the human diet. Endogenously they are the main responsible for physiological and ecological functions and it has been observed that their concentrations vary in situations of biotic and abiotic stress (Frontela *et al*., 2010; Sauceda *et al*., 2011). Therefore, it is important to recognize the dynamics of these compounds associated with stress and environmental variation, since this information can serve as an indicator to develop management strategies for their best use (Qasim *et al*., 2017).

Especially in the case of species of food interest and spices, since variations in the contents of phenolic compounds and their antioxidant and antiradical activities can be considered as indicative parameters of quality and potential benefits through their consumption (Capecka *et al*., 2005; Ninfali *et al*., 2005; Cobaleda-Velasco *et al*., 2017).

Recently, it has been pointed out that *Portulaca oleracea* L., is a good source of beneficial compounds for human health, including omega-3 and β-carotene fatty acids, vitamins, essential amino acids, α-tocoferol, mucilages, antioxidant compounds such as glutathione and ascorbic acid, phenolic compounds and minerals (Oliveira *et al*., 2009; Ramadan *et al*., 2017; Khodadadi *et al*., 2018; Allahmoradi *et al*., 2018). This plant is considered a weed of almost worldwide distribution, although in many regions it is appreciated for its nutritional and medicinal properties.

Recently, the antioxidant potential of *P. oleracea* has been considered as a criterion for its consumption and use, so the exploration of accessions that have considerable levels of these compounds represents an opportunity to study the variations of these compounds in breeding plans (Alu'datt *et al*., 2019; Habibian *et al*., 2020). In Mexico, this plant is consumed in various ways and its wide distribution and plasticity has been highlighted to adapt to different environments (Gutiérrez *et al*., 2007; Alam *et al*., 2014; Román *et al*., 2018).

Regarding the accumulation of antioxidant components, it has been observed that many plants vary their phytochemical composition depending on environmental and growth conditions (Bautista *et al*., 2016; Caverzan *et al*., 2016), which can compromise its quality for food or medicinal purposes (Bartolini *et al*., 2018). These fluctuations have been observed in cultivated and wild varieties of *P. oleracea*, where it is highlighted that the cultivated varieties have higher content of phytochemicals and antioxidant activities (Nemzer *et al*., 2020; Sdouga *et al*., 2020).

However, it must be considered that the wild forms of some species may be reservoirs of diverse compounds in different proportions than those cultivated due to the influence of environmental conditions and genetic characteristics (Mocan *et al*., 2017). In the northeastern part of Mexico, the consumption and cultivation of *P. oleracea* is quite limited, even though it is a plant of wide distribution, associated with settlements and agricultural areas. This plant represents a food resource that is not exploited and that has also shown to have the potential to supplement the diet with antioxidant compounds.

In this sense, the nutrimental and nutraceutical exploration of the populations of this species has been suggested to determine their responses to environmental conditions to develop strategies that allow the selection and use of this natural resource.

Therefore, the objective of this research was to determine the variation of the contents of phenolic and antioxidant compounds in accessions of *P. oleracea* grown in five municipalities of the central part of Tamaulipas where populations were located growing in uncultivated form, in order to contribute to the knowledge of the plant material of the region and suggest its use.

Materials and methods

Collection of plant material

The plant material of *P. oleracea* was collected in the municipalities of Abasolo, Jiménez, Llera, Padilla and San Nicolás, Tamaulipas, Mexico (Table 1). The species was identified in the herbarium of the Autonomous University of Tamaulipas. The sites were selected for the presence of the plant, which had not been previously reported, in addition the agricultural activity in these areas is carried out with little application of agrochemicals.

Table 1. Geographical location of each accession of *P. oleracea* in zone 14 given in UTM, the climate type given by the Köppen classification (García, 2004).

Accession	Coordinates	Climate	Municipality
AB	2660772.51 N, 563071.21 E	BSh	Abasolo
JN	2677473.20 N, 52204.40 E	BSh	Jiménez
LL	2578673.3 N, 497649.70 E	BSh	Llera
PA	2687973.20 N, 50208.40 O	Cfa	Padilla
SN	2731097.59 N, 517130.63 E	BSk	San Nicolás

Preparation of extracts

The collected material was washed with running water to remove soil particles and plant debris, then dried at 50 °C for 96 h in a forced air furnace. The dry material was pulverized with an electric mill and sieved through a 700 μm mesh (US Standard sieve). The plant material was subjected to extraction with three solvents, for the extraction with ethanol and acetone a 1:4 (w/v) ratio was used and for the extraction with distilled water a 1:6 (w/v) ratio was used.

The mixture of tissue and solvents was stirred for 20 min and then 10 centrifuged at 8 000 x g for 10 min. The supernatant was recovered and used as a source of phenolic compounds for subsequent determinations. The extracts were stored at -20 °C until use.

Detection of total phenolic compounds (CFT)

Total phenolic compounds were quantified with the Folin-Ciocalteu reagent (Singleton *et al*., 1999), using a standard curve prepared with gallic acid (Sigma-Aldrich, St. Louis, MO, USA) with a range of 100 to 500 μg ml^{-1}. The reactions were prepared by mixing the samples with 250 μl of the Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA) and 750 μl of 20% Na_2CO_3, then incubated in darkness for 2 h at 37 °C. Subsequently, the absorbance was recorded at 765 nm. Results were reported in milligrams of gallic acid equivalents per gram of tissue in dry weight (mEAG g^{-1} PS).

Detection of scavenging capacity against the DPPH free radical

For the inhibition of the DPPH free radical (2,2 diphenyl-1-pricrylhydrazyl), the method reported by Brand-Williams *et al*. (1995) was used, 50 μl of the extracts were mixed with 2.95 ml of the DPPH reagent (Sigma-Aldrich, St. Louis, MO, USA) at 60 μM and left to react for 30min, simultaneously, a standard curve was prepared with Trolox (Sigma-Aldrich, St. Louis, MO, USA) in a range of 100 to 1 200 μM and they were made to react in the same way as the samples with the DPPH. After incubation, all samples were read at 517 nm and absorbance was used to calculate the concentration according to the standard curve. The results were expressed as mM of Trolox equivalents per gram of dry weight (mM ET g^{-1} PS).

Detection of scavenging capacity against the ABTS free radical

The scavenging capacity against the ABTS free radical (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) was evaluated according to the method reported by Re *et al*. (1999). A concentrated solution was prepared with 7 mM of ABTS (Sigma-Aldrich, St. Louis, MO, USA) and 2.45 mM of potassium persulfate (Sigma-Aldrich, St. Louis, MO, USA), which was left to stand for 12 h in the dark to activate it. Subsequently, this solution was adjusted with ethanol to reach an absorbance of 0.7 at 734 nm. The extracts in the presence of the ABTS reagent were incubated for 6 min in the dark and subsequently their absorbance was recorded, this was compared with a standard curve with Trolox (Sigma-Aldrich, St. Louis, MO, USA) in a range of concentrations of 100 to 1 200 μM. The scavenging capacity against ABTS was calculated and expressed as mM ET g^{-1} PS.

Statistical analysis

Three extractions were made with each solvent for each accession and all extractions were determined in triplicate. To normalize the data, all were transformed with the box-cox function in the statistical program PAST (Hammer *et al*., 2001). An analysis of variance was performed followed by a comparison of means with the Tukey test (α= 0.05) in those treatments where statistically significant differences were found.

Results

All accessions had detectable levels of the three parameters evaluated. When comparing the global contents extracted in a general way for each of the parameters evaluated by municipalities, it was observed that only in the case of CFT there was a slight difference in contents, in the case of the accession of the municipality of Llera. On the other hand, the activity against ABTS and DPPH did not show significant differences between accessions (Table 2). The detection of CFT and phytochemicals with antioxidant properties in the different accessions of purslane showed significant differences in the three parameters evaluated ($p \leq 0.05$) in the case of the solvent used in the extraction process, which allows to recognize the differential obtaining of the phytochemical components according to their polarity (Table 3).

Table 2. Comparison of means of the global contents of total phenolic compounds CFT, free radical scavenging activity against ABTS and DPPH in the different accessions.

Municipality	CFT (mEAG g^{-1} PS)	ABTS (mM ET g^{-1} PS)	DPPH (mM ET g^{-1} PS)
AB	3.9797 ±1.48 a	1.9642 ±1.01 a	1.4643 ±0.41 a
JN	3.88841 ±0.3 a	2.268 ±0.71 a	1.5164 ±0.28 a
LL	2.4383 ±0.81 ab	2.3492 ±0.55 a	1.4852 ±0.25 a
PA	3.1377 ±1.95 a	1.9025 ±1.07 a	1.4299 ±0.35 a
SN	3.758 ±0.61 a	2.0682 ±0.73 a	1.4615 ±0.32 a

Different letters in the same column indicate statistically significant differences between accessions ($p \leq 0.05$).

The solvent used significantly influenced the parameters analyzed. When the general comparison of the levels of the parameters evaluated in relation to the solvent was made, the difference of the organic solvents with respect to the levels extracted with the water was noticeable. Water was the solvent that allowed the largest quantity of compounds to be extracted. For the three parameters, acetone and ethanol extracted statistically similar levels (Table 3).

Table 3. Comparison of means of the global contents of total phenolic compounds, free radical scavenging activity against ABTS and DPPH extracted with different solvents.

Solvent	CFT (mM ET g^{-1} PS)	ABTS (mM ET g^{-1} PS)	DPPH (mM ET g^{-1} PS)
Acetone	2.9114 ±0.75 b	1.4561 ±0.57 b	1.2618 ±0.13 b
Water	4.5781 ±0.97 a	3.1385 ±0.1 a	1.894 ±0.09 a
Ethanol	2.7769 ±1.15 b	1.686 ±0.22 b	1.2341 ±0.08 b

Different letters in the same column indicate statistically significant differences between accessions ($p \leq 0.05$).

The contents of CFT in each accession with respect to the solvent acetone showed that the highest levels were extracted in the AB and SN accessions, while the lowest with this solvent were LL and PA. In the case of the highest content recorded with the solvent water, it was the accessions AB and PA, while the lowest was LL. The highest content of CFT using ethanol for extraction was with the JN accession and the lowest for the PA accession (Table 4). Heterogeneous profiles were observed with respect to CFT levels depending on the solvent, most accessions showed the highest levels with water, except JN.

Table 4. Comparison of the contents of the total phenolic compounds (CFT) (mEAG g^{-1} PS) quantified in the samples of purslane (*P. oleracea*) extracted with different solvents.

Accession	Acetone	Water	Ethanol
AB	3.2678 ±0.11 d	5.8938 ±0.03 a	2.6984 ±0.61 f
JN	3.0226 ±0.09 e	3.7228 ±0.22 c	4.2194 ±0.16 b
LL	2.0944 ±0.48 g	3.4291 ±0.4 cd	1.7914 ± 0.2 gh
PA	1.9959 ±0.45 g	5.3742 ±0.11 a	1.2409 ±0.45 h
SN	3.2104 ±0.29 d	4.4707 ±0.21 b	3.5929 ±0.37 cd

Different letters in the same column indicate statistically significant differences between accessions ($p \leq 0.05$).

The highest free radical scavenging capacity against ABTS were obtained using water as a solvent, with AB and JN accessions having the highest content. On the other hand, the highest value reached during the extraction with acetone was that of the LL accession, higher than the content obtained in the ethanol extracts and, on the contrary, the lowest value against ABTS was recorded in the PA accession (Table 5).

Table 5. Comparison of the contents of free radical scavenging activity against ABTS (mM ET g^{-1} PS) quantified in the samples of purslane (*P. oleracea*) extracted with different solvents.

Accession	Acetone	Water	Ethanol
AB	0.9539 ±0.28 h	3.27 ±0.06 a	1.5565 ±0.09 fg
JN	1.6494 ±0.23 f	3.2226 ±0.04 a	1.9321 ±0.04 d
LL	2. 2076 ±0.06 c	3.069 ±0.01 b	1.7711 ±0.05 de
PA	0.8135 ±0.02 i	3.099 ±0.07 b	1.2974 ±0.12 g
SN	1.3862 ±0.28 g	3.032 ±0.07 b	1.7863 ±0.06 de

Different letters in the same column indicate statistically significant differences between accessions ($p \leq 0.05$).

The levels of scavenging capacity against the DPPH radical showed that the LL accession was the one with the highest content when acetone was used as a solvent and the lowest was PA. In the case of water as a solvent, it was observed that the AB accession was the highest and the LL accession the lowest. In the case of ethanol, the JN accession was the one that presented the highest level of scavenging capacity while PA had the lowest levels (Table 6).

Table 6. Comparison of the contents of free radical scavenging activity against DPPH (mM ET g^{-1} PS) quantified in the samples of purslane (*P. oleracea*) extracted with different solvents in the different municipalities of study.

Accession	Acetone	Water	Ethanol
AB	1.1457 ±0.04 g	2.0204 ±0.05 a	1.1915 ±0.02 fg
JN	1.2803 ±0.05 e	1.9038 ±0.04 b	1.3651 ±0.03 de
LL	1.4526 ±0.09 d	1.7963 ±0.04 c	1.2068 ±0.06 fg
PA	1.1174 ±0.08 gh	1.8411 ±0.08 bc	1.169 ±0.06 g
SN	1.2521 ±0.08 e	1.9087 ±0.04 b	1.2238 ±0.04 f

Different letters in the same column indicate statistically significant differences between accessions ($p \leq 0.05$).

The above shows that there is an important heterogeneity in relation to each accession and with respect to the polar nature of the phenolic components and the free radical scavenging capacities in all accessions. Water was the solvent with the highest extraction capacity compared to evaluated organic solvents. Only the JN accession had the highest CFT levels with ethanol.

Discussions

The levels of CFT and the free radical scavenging capacity in the accessions of *P. oleracea* evaluated in the present work allow the observation of a marked heterogeneity when compared under the same extraction condition. Although the origin of the samples corresponds to different and distant growth zones, in-depth studies are still lacking to determine the effect of environmental conditions on the levels of each parameter, although the literature indicates that these could influence the balance of antioxidants and phenolic compounds, being considered as adaptive responses to stressful situations (Gharibi *et al*., 2016; Kaur *et al*., 2017).

The levels of CFT and antioxidants are directly related to signaling processes in plants, as well as defense against herbivores, protection against light stress, tolerance of heavy metals and to protect the redox balance of plants; therefore, their levels will depend on the conditions where the plants develop. The contents detected in this study are similar to those reported by previous studies (Santiago-Saenz *et al*., 2018; Sicari *et al*., 2018; Habibian *et al*., 2020) although, the chemical identity of the agents responsible for antioxidant effects is unknown in the accessions from Tamaulipas.

The nature of the solvent used during extraction allowed for a more complete exploration of the contents of phenolic and phytochemical compounds with free radical scavenging activity in wild accessions, since it has been observed that the solvent has a strong influence on the diversity and concentrations obtained from phytochemicals with antioxidant activities, which is attributed to the effect of polarity (Felhi *et al*., 2017; Thouri *et al*., 2017). This coincides with the results showing that most accessions of *P. oleracea* had higher contents in aqueous extracts and some differentially between acetone and ethanol.

This influence of the solvent on the levels and nature of the extracted phytochemicals has been previously reported in *P. oleracea* (Habibian *et al*., 2020), where it is also noted that the contents of CFT and the levels of scavenging capacity against ABTS and DPPH were similar between ethanolic and aqueous extracts, as occurred for most of the accessions in this study. It has been reported that solvents with higher polarity allow the extraction of higher CFT contents and free radical scavenging capacity, this explains why water was more efficient in extracting these metabolites of *P. oleracea* (Almulaiky *et al*., 2020).

The characterization of wild varieties of purslane and the knowledge of the optimal growing conditions are key aspects during the management processes. Because wild forms constitute reservoirs of genetic information and physiological responses that could at some point be transferred from wild relatives to cultivated forms. Either through genetic improvement or modulated through the modification of environments, so that the accumulation or decrease of a given phytochemical is favored (Van Treuren *et al*., 2018; Araghi *et al*., 2019).

Variation in primary and secondary physiological aspects is a phenomenon already reported for *P. oleracea*, where wild accessions respond differentially to regeneration, evapotranspiration, photosynthesis and chlorophyll content (Alam *et al*., 2014). It has also been pointed out that there is a high variability in the accumulation of compounds of phenolic nature between varieties of *P. oleracea*, something that coincides with what was observed in the accessions of this work. This variation should be considered as an important part for the management of *P. oleracea* because it could compromise the contents of compounds of nutritional importance and bioactive compounds (Sdouga *et al*., 2020).

The contents of CFT observed in the accessions of *P. oleracea* detected in this work are lower than the values reported in previous studies, which vary between 5.57 and 21.61 mg EAG g^{-1} PS, which could be associated with the type of extraction used and the polarity of the solvent used (Gallo *et al*., 2017; Gatea *et al*., 2017). Regarding the influence of polarity on the extracted levels, from *P. oleracea*, it has been reported that hydroalcoholic mixtures with contents of 30 and 50% have better yields than water alone or absolute ethanol (Gallo *et al*., 2017), which opens the possibility that in the tissues of the accessions from Tamaulipas there could be values that were not detected due to the influence of the solvent.

Regarding the contents of scavenging capacity against the ABTS radical, levels higher than those reported in previous studies were detected, where contents between 4.71 and 74.6 μM of ET g^{-1} PS are indicated, in which the influence of the growth conditions and the nature of the solvent used were also mentioned, highlighting that the ethanol phases at 30 and 50% were more efficient in the extraction (Gatea *et al*., 2017; Santiago-Saenz *et al*., 2018).

In the case of the scavenging capacity against the DPPH radical, the values recorded in this study were higher than that reported by Santiago-Saenz *et al*. (2018), when comparing the contents of *P. oleracea* with quelite cenizo (*Chenopodium berlandieri* L.) and quintonil (*Amaranthus hybridus* L.), the purslane and the quelite cenizo had the highest contents of CFT and scavenging capacity against ABTS. *P. oleracea* had higher scavenging capacity against DPPH, while the quintonil was the lowest with respect to the three parameters indicated (Santiago-Saenz *et al*., 2018). This points to *P. oleracea* as an important source of compounds with potential antioxidant capabilities that could give added value to this plant as a food material.

When comparing the contents of the parameters evaluated in the accessions of *P. oleracea* from Tamaulipas, it was observed that all had CFT contents lower than those of various plants used as condiments; however, regarding the levels of scavenging capacity against ABTS, they are within the ranges reported for 39 spices used as condiments.

In the case of the values recorded for the scavenging capacity against DPPH of wild accessions, this is in ranges similar to the contents reported for species of culinary and medicinal use (Chan *et al*., 2016; Assefa *et al*., 2018). The results of the present study indicate the nutritional and functional potential of the wild purslane from Tamaulipas, since it shows contents of compounds with free radical scavenging capacity equivalent to those of several edible plants or medicinal use, which reinforces its potential for consumption.

It is important to note that the variation found in accessions could be attributed to climatic and growth conditions; however, more in-depth studies are needed to designate which parameters have the most influence on these variations. It should also be considered that, as they are wild accessions, they must be evaluated to determine parameters of importance in nutrition as part of the knowledge necessary for the use of this species for food and nutraceutical purposes or for management plans of the species.

Conclusions

The wild accessions of *P. oleracea* in the state of Tamaulipas presented a variation in the levels of phenolic compounds and in the free radical scavenging capacity, in these, the type of solvent had an influence on its extraction. These parameters are important and must be considered for the use of this natural resource and the development of products for human consumption.

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