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Phenolic compounds and their antioxidant activities in *Portulaca oleracea* L. related to solvent extraction

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Abstract

Our work aims to explore and compare the biological evaluations of *Portulaca oleracea* leaves by the study of contents in phenolic compounds and antioxidant capacities in order to support scientifically its traditional interest using different solvent for optimization of the extraction. The first part of this study concerns the successive Soxhlet extraction and quantification of total phenols, flavonoid and condensed tannin by the reagent Folin-Ciocaleu, the aluminum trichloride and the vanillin test, respectively. The second part is the study of the antioxidant activities of plant extracts by using two *in vitro* methods: evolution of total antioxidant capacity and quenching of radical DPPH. The results showed that the methanol fraction is very rich in total polyphenols (13.41 EAG mg / g DM) and exhibits a good antioxidant activity especially for scanning free radicals (IC50 = 78.44 mg/ml). These results are encouraging for the recovery of this species as an excellent medicinal and alimentary plant.

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Introduction

In the severe climatic conditions, the plants produce a reactive oxygen species (ROS) which leads to cellular damages and they are known to be implied in several plant disruptions. The ROS could react also with biological molecules, such as the DNA, proteins or liquids are generating the mutations and damaging the membranes leading to cellular damages at the cells and tissue level (Abdi and Ali, 1999). The biotic and abiotic factors exert a significant influence on the secondary metabolite range particularly on the phenolic compounds (Ksouri *et al.*, 2007).

This range of phenolic compounds are so variable much on qualitative than quantitative plan. These variations are controlled by genetic factors, plant development and environmental conditions (De Abreu and Mazzafera, 2005).

The strengthening of secondary metabolite synthesis as a response to stress condition is supposed to protect the oxidation cellular structures (Chanwitheesuk *et al.*, 2005). Indeed, the adaptation of numerous plant species in hostile environmental conditions suggests the antioxidant presence in their tissues (Maisuthisakul *et al.*, 2007; Jaleel *et al.*, 2008).

The antioxidants are divided into two principle types according to their actions. Primary antioxidants can inhibit or delay the oxidation by trapping the reactive species of oxygen through the reduction. The secondary antioxidants which function by metallic ions connection, the hydroproxides to non-radical species absorbing the UV radiations or the deactivation of the singulet oxygen. Among the different types of natural antioxidants, the polyphenols are those that have more attention (Luo *et al.*, 2002).

In terms of structure, the phenolic compounds include aromatic cycle, taking one or more substituent hydroxyl, and the range of phenolic molecules goes from the simple to the highly polymerized compound (Sakihama *et al.*, 2002). The phenolic acids, flavonoids and tannins are considered as the main food phenolic compounds. In addition to this diversity, the polyphenols can be associated with various carbohydrates and organic acids (Manach *et al.*, 2004). These compounds have a wide range of physiological properties, such as the anti-allergic effects, anti-atherogenic, anti-inflammatory, anti-microbial, anti-oxidant, anti-thrombotic, cardioprotective (Balasundram *et al.*, 2006). There is an interest move toward the natural antioxidants for their use in medicinal food and matter to substitute the synthetic antioxidants which are restrict because of their carcinogenicity suspected (Velioglu *et al.*, 1998). Moreover, the natural compounds have an antioxidant activity stronger than that of synthetic compounds (Maisuthisakul *et al.*, 2007).

Portulaca oleracea is an annual summer plant that sprouting in the natural state in the entire Mediterranean basin especially in the hot regions, it belongs to Portulacaceae family. This specie is known for its traditional use against kidneys and digestive troubles, against the Pyrosis, the spitting of blood, persistent coughs, insomnia and in varied chronic diseases where it acts as a softner.

In Algeria there are few published works on the characterization of these bioactives (polyphenols). This work deals with this aspect to provide a decision making tool for the development and the improvement of this specie in order to valorize it in the therapy domains, medicinal and agro-alimentary.

Materials and methods

Preparation of samples

Portulaca oleracea came from Touggourt region (south-east Algeria), was collected in August 2014, then the aerial part dried in the shade. The leaves finely crushed/grined using blade crushers then they are conserved in glass flasks in the obscurity at 4°C until analysis.

Sample extraction

The aerial parts of *Portulaca oleracea* were extracted by four solvents of growing polarity, namely the hexane (polarity = 0.0), ethyl acetate (polarity = 4.4), the methanol (polarity = 5.1) finally water is being

considered as the most polar solvent. 30 g of fine powder of purslane are placed in the cartridge of the soxhlet with 300 ml of solvent. Each extraction lasts 24 hours at which the extract is recovered. Thus the recovered extracts are conserved in the obscurity to 4°C.

Determination of total polyphenol content

Total polyphenol content (TPC) was determined according to the method of Singleton and Rossi (1965) with some modifications. An aliquot of diluted sample fraction was added to 0.5 ml distilled water and 0.125 ml Folin-Ciocalteu reagent. The mixture was shaken and incubated for 6 min before adding 1.25 ml Na_2CO_3 (7%). The solution was then adjusted with distilled water to a final volume of 3 ml and mixed thoroughly. After incubation in the dark, the absorbance was read at 760 nm versus a prepared blank. Total phenolic contents were expressed as milligrams gallic acid equivalents per gram dry residue (mg GAE/ g DW) through the calibration curve with gallic acid. All samples were analyzed in triplicates.

Determination of total flavonoid content

Total flavonoid content (TFC) was measured by a modified aluminium chloride colorimetric assay, described by Liu *et al.* (2008). An aliquot of diluted sample or standard solution of (+)- catechin was added to 75µl $NaNO_2$ solution (7%) and mixed for 6 min before adding 0.15 ml $AlCl_3$ (10%). After 5 min, 0.5 ml NaOH solution (1 M) was added. The final volume was adjusted to 2.5 ml, thoroughly mixed, and the absorbance of the mixture was determined at 510 nm. Total flavonoid were expressed as mg (+)-catechin equivalent per gram dry residue (mg CE/g DW), through the calibration curve of (+)- catechin (0–400 µ g ml^{-1} range). All samples were analyzed in triplicate.

Determination of total condensed tannin

Procyanidins were measured using the modified vanillin assay described by Sun *et al.* (1998). Three milliliters methanol vanillin solution (4%) and 1.5 ml concentrated H_2SO_4 were added to 50 µl sample. The mixture was allowed to stand for 15 min and the absorbance was measured at 500 nm against methanol as a blank. The amount of total condensed tannin was expressed as mg CE/g DW. All samples were analyzed in triplicate.

Determination of antioxidant assays

Determination of total antioxidant activity

Total antioxidant capacity was evaluated through the assay of a green phosphate/Mo^{5+} complex according to the method described by Prieto *et al.* (1999). An aliquot (0.1 ml) of diluted extract/fraction was combined with 1 ml reagent solution (0.3 N sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). Ethanol and water were used instead of sample for blank. Mixtures were incubated in a boiling water bath for 90 min then cooled to room temperature. Their absorbance was measured at 695 nm against blank. Antioxidant capacity was expressed as mg gallic acid equivalent per gram dry residue (mg GAE/g DW). All samples were analyzed in triplicates.

Determination of DPPH radical scavenging activity

The 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical scavenging activity was determined by the method of Brand-Williams *et al.* (1995) with slight modifications. One milliliter of the extract at known concentrations was added to 0.5 ml of a DPPH methanolic solution. The mixture was shaken vigorously and left standing at room temperature in the dark for 30 min. The absorbance was then measured at 517 nm and corresponds to the extract ability to reduce the radical DPPH to the yellow-colored diphenylpicrylhydrazine. BHT was a synthetic phenolic used as positive standard. The antiradical activity was expressed as IC_{50} (µg/ml), the antiradical dose required to cause a 50% inhibition. The ability to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging effect (%) = $[(A_0 - A_1)/A_0]_* 100$

Where A_0 is the absorbance of the control at 30 min, and A_1 is the absorbance of the sample at 30 min. All samples were analyzed in triplicates.

Statistical analysis

All analyses were done in triplicates. Results were expressed as means ± standard deviations. The data were statistically analysed using the Minitab.2000 statistical software. An independent *t*-test was used for comparison of means between groups. One-way analysis of variance (ANOVA) is used to compare means for each studied parameters. The level of significance was set at $p < 0.05$.

Results

Quantification of total polyphenol, flavonoid and condensed tannin

the quantitative analyzes of total polyphenol, total flavonoid and condensed tannin are determined from the equations of the linear regression of each calibration curve expressed successively in milligram equivalent of gallic acid and milligram equivalent of catechin per gram of dry weight.

The obtained results are presented in Table 1.

Table 1. Total polyphenol (mg GAE/ g DW), flavonoid and condensed tannin (mg CE/g DW) in *Portulaca oleracea* for ethyl acetate, methanol and water extracts.

	Extraction solvent		
	Ethyl acetate	Methanol	Water
Total polyphenol	0,751±0,076	13,401±1,419	1,970 ±0,081
Flavonoid	0,466±0,005	4,413±0,150	2,690±0,030
Concensed tannins	0,080±0,010	0,396±0,058	0,346±0,070

We have registered a significant variability according to the solvent. We notice from table 01 that the amount of the phenolic compounds varies between 0.751 and 13.401 mg GAE/g DW. The highest level of phenolic compounds was detected in the methanolic extract, it is 7 and 17 times higher than those reported in the aqueous extracts and ethyl acetate.

We note from table 1, that the amount of flavonoid varies between 0.466 and 4.413 mg CE/g DW. The highest rate of flavonoid was detected in the methanol extract, on the contrary the amount registered for the ethyl acetate extract remained the lowest. The methanolic extract is 1.5 and 9 times richer than the aqueous extracts and ethyl acetate respectively.

Concerning Condensed tannin, we note from results in table 01 that the amount of the condensed tannin varies between 0.080 and 0.396 mg CE/g DW.

The rates of condensed tannin in the methanolic and aqueous extracts are statistically identical. These two extracts are 4 times richer in these compounds than the ethyl acetate extract with 0.080 mg CE/g DW.

Evaluation of antioxidant activities

In this work, the antioxidant activity of the extracts was evaluated *in vitro* by two different methods: total antioxidant activity and free radical test DPPH (Table 2).

Table 2. Total antioxidant capacity (mg GAE/ g DW) and DPPH radical scavenging (µg/ml) in *Portulaca oleracea* for ethyl acetate, methanol and water extracts.

	Extraction solvent		
	Ethyl acetate	Methanol	Water
Total antioxidant capacity	0,556±0,030	2,833±0,332	0,900±0,155
DPPH	114,84±22,81	78,44±7,24	89,42±6,63
BHT	11.5	11.5	11.5

Results in table 02 show that total antioxidant capacities were very variable from one extract to another. The methanolic extract has the best total antioxidant capacity which is in the range of 2.833 mg GAE/ g DW following by the aqueous extract and at last ethyl acetate. The methanol extract is 3 and 5 times more powerful than the aqueous and ethyl acetate extracts respectively.

The values of free radical test DPPH (Table 2) show that the IC_{50} vary widely between the different solvents and are included between 89.42 and 114.84 µg/ml. Among the three fractions of our plant, the methanol fraction represents the most active extract with an IC50 in the range of 78.44 $\mu g.ml^{-1}$, followed by the aqueous extract and finally the lowest antiradical activity was expressed by the ethyl acetate extract.

Table 3. One-way (solvent) analysis of variance.

Analysis of Variance for Polyphenol

Source	DF	SS	MS	F	P
Regression	2	292,178	146,089	216,26	0,000
Error	6	4,053	0,676		
Total	8	296,232			

Analysis of Variance for Flavonoid

Source	DF	SS	MS	F	P
Regression	2	23,48927	11,74463	1495,07	0,000
Error	6	0,04713	0,00786		
Total	8	23,53640			

Analysis of Variance for Condensed tannins

Source	DF	SS	MS	F	P
Regression	2	0,17389	0,08694	30,81	0,001
Error	6	0,01693	0,00282		
Total	8	0,19082			

Analysis of Variance for total Antioxidant capacity

Source	DF	SS	MS	F	P
Regression	2	9,0389	4,5194	99,79	0,000
Error	6	0,2717	0,0453		
Total	8	9,3106			

Analysis of Variance for DPPH

Source	DF	SS	MS	F	P
Regression	2	2091	1046	5,08	0,051
Error	6	1234	206		
Total	8	3325			

DF : degree of freedom ; SS : sum of square ; MS : mean square

Discussion

In this section, the phenolic compounds contents and the antioxidant activities *in vitro* were investigated. The obtained results showed an important intraspecific variability for the phenolic content and antioxidant activities.

Quantification of total polyphenol, flavonoid and condensed tannin

The results of the total polyphenol content, flavonoid and condensed tannin contents show that the methanol still the richest extract in comparison with aqueous and ethyl acetate.

In this context, Hayouni *et al.* (2007) showed that the polar fractions contain more phenolic compounds than the less or nonpolar fractions.

These data are also confirmed by recent works by Fernandez-Agullo *et al.* (2013), which proved that the phenolic contents decrease considerably with the decrease in solvents polarity. This explains partly the obtained results in our work where the ethyl acetate fraction remains very poor in these molecules.

The solubility of the phenolic compounds depends on their degree of polymerization, the interaction with

the other constitutes and the type and effect of the solvent used or even the used extraction method. In this context, Jallali *et al.* (2012) reported that the flavonoid contents of *Crithmum maritimum* extracts by simple maceration (4.77 mg CE/g DW) were higher than those found by the soxhlet system (1.87 mg CE/g DW).

The distribution of the secondary metabolites can change during the development of the plant. This can be related to hard climatic conditions (high temperature, solar exposure, drought, salinity), which stimulate the biosynthesis of the secondary metabolites such as polyphenols (Falleh *et al.*, 2008). However, the phenolic content of a plant depends on some number of genotypic factors (El-Waziry and Ibrahim, 2007), biotic conditions (species, organ and physiological stage) and abiotic factors (edaphic factors) (Ksouri *et al.*, 2008), the nature of the soil and the microclimate type (Atmani *et al.*, 2011) and also the bioclimatic stages in which these plants could grow.

The total polyphenol, flavonoid and condensed tannin contents found by Falleh *et al.* (2009), in *Mesembryanthemum crystallinum* are 1.43 mg GAE/g DW, 0.31 mg GAE/g DW and 0.06 mg GAE/g DW respectively. This species is known for its medicinal interest but it still all the same that our results widely overtake those observed in *Mesembryanthemum crystallinum*.

Proestos *et al.* (2006) who estimated the total polyphenol content of 14 aromatic and medicinal species, reported total polyphenol contents varying from 0.2 to 42 and 2.9 to 28.2 mg GAE/g DW respectively. This allows us to set the purslane among the plants quite rich in total polyphenol.

On the other hand, our results are much better by referring to previous works done on recognized medicinal plants. In this meaning, Saadaoui *et al.* (2007) showed that the polyphenol content of the methanol extract of some plants belonging to different families *Punica gramatum*, *Retama retam*, *Thymus capitatus*, *Rosmarinus officinalis*, *Ruta chalepensis*, *Ajuga iva*, *Lawsonia inermis* and *Agave americana* varies between 1.68 to 11.07 mg GAE/g DW, which puts our plant in pole position.

Evaluation of antioxidant activities

As for phenolic compounds, the main results showed a significant variability of the total antioxidant capacity between the three extracts of the studied species. This capacity is higher for the methanol extracts in comparison with aqueous and ethyl acetate. The antioxidant activity registered according to the extraction solvent is classified in the following order: Methanolic extract> aqueous extract> ethyl acetate extract.

More generally, methanol extracts show very important anti-radical activities and are distinguished by their high total antioxidant capacity (53.7 mg GAE/g DW), which would be due to the presence of the phenolic compounds. The literature data shows generally a positive correlation between the total polyphenol content of an extract and its antioxidant power (Li *et al.*, 2009; Liu *et al.*, 2011). This preferential distribution of the phenolic compounds in the aerial parts is probably is originally of their strong antioxidant activity. This could explain the low antiradical activity of the ethyl acetate extract compared to the other extracts and which would be related to a lower content of total polyphenol (0.751 mg GAE/g DW). Other authors showed that the methanol remains the best solvent to extract antioxidants from a plant (Sun *et al.*, 2007).

Consequently, these capacities are probably correlated with their contents of phenolic compounds in particular in flavonoid and condensed tannin since some studies confirmed the close relation between the contents of these secondary metabolites and the antioxidant activities, particularly the polyphenol contents. Several results affirmed that the Proanthocyanidins constitute antioxidants power that play a nutritional, physiological and pharmacological role (De Rezende *et al.*, 2009 ; Diouf *et al.*, 2009).

Subhasree *et al.* (2009) confirmed in a study of two glycophyte plants *Pisonia alba* and *Centella asiatica*

that the antioxidant activity of these plants is lower than that found in plant note with IC_{50} values of 175 and 200 µg/ml , respectively.

Kang and Saltveit (2002) suggested that plant extracts that contain polar molecules show high antiradical activity. This explains in part the low activity in the ethyl acetate extracts.

On the other hand, antioxidant activities are more variable in the species of the plant (inter-species) than in the same species (intra-species) (Ksouri *et al*., 2008).

In summary, the capacities for trapping the radical are classified in the following order: Methanolic extract> aqueous extract> ethyl acetate.

All of these *in vitro* results are only a first step in the search for biologically active substances of natural origin, an *in vivo* study is preferable to obtain a deep view of the antioxidant activities of this plant.

Conclusion

Through our work we studied the phenolic composition of the aerial part through a quantitative evaluation by determining the contents in total polyphenol, flavonoid and condensed tannin, and we tested the antioxidant efficiency of this species with two *in vitro* methods, total antioxidant activity and DPPH test.

According to the obtained results, the phenolic compounds of the purslane are moderately polar since the methanolic extract is the richest in these compounds while demonstrating the best antioxidant activities. Our results show that the methanolic fraction of the purslane is quite rich in total polyphenol with a content of 13.4 mg GAE/g DW and flavonoid with a concentration of 4.41 mg CE/g DW. This extract also showed a good free radical activity against the free radical DPPH with an IC50 equal to 78.44 µg/ml.

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