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## RESEARCH ARTICLE

# Variation in phenolic compounds, $\alpha$ -linolenic acid and linoleic acid contents and antioxidant activity of purslane (*Portulaca oleracea* L.) during phenological growth stages

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**Abstract** Purslane (*Portulaca oleracea* L.) contains a variety of natural products with different biological properties. The objective of this research was to estimate changes in total phenolics, flavonoids, and fatty acids ( $\alpha$ -linolenic acid and linoleic acid) contents as well as antioxidant activity of *P. oleracea* at different growth stages. Seeds were germinated in soil-filled plastic pots at greenhouse. Leaves and stems were collected at the vegetative and flowering stages. Total phenol and flavonoid contents of the samples were determined by Folin–Ciocalteu and aluminum chloride methods, respectively. The contents of  $\alpha$ -linolenic and linoleic acids were determined using gas chromatography analysis after transesterification of fatty acids. Furthermore, Ferric reducing antioxidant power and 1,1-diphenyl-2-picrylhydrazyl assays were used to determine the antioxidant activities. The highest contents of total phenols (698.6 mg GAE 100 g<sup>-1</sup> DW), flavonoids (46.9 mg QE 100 g<sup>-1</sup> DW),  $\alpha$ -linolenic acid (2.7 mg g<sup>-1</sup> DW) and linoleic acid (0.8 mg g<sup>-1</sup> DW) were determined in the leaves at flowering stage. Free radical scavenging capacity was significantly affected ( $P \leq 0.05$ ) by age; and the leaves of purslane had more antioxidant potential compared to stems. A positive correlation was observed between the antioxidant activities and total phenols content. Overall, purslane leaves at flowering stage can be regarded as a valuable source of fatty acids (especially  $\alpha$ -linolenic acid) and antioxidants in human diet.

**Keywords** DPPH Fatty acids FRAP · Omega-3 · Plant extracts

## Introduction

Purslane (*Portulaca oleracea*) is an annual medicinal plant from the family of *Portulacaceae*. As a native plant of India and Middle East, purslane is rich in polyunsaturated fatty acids (PUFAs) including omega-3 ( $\alpha$ -linolenic acid; C18:3) and omega-6 (linoleic acid; C18:2) fatty acids (Marcela Eljach Mosquera 2013; Azuka et al. 2014). The highest omega-3 fatty acid contents have been found in purslane as compared to other vegetables (Azuka et al. 2014). The other constituents of the plant include glutathione (Lalromawii Devi et al. 2014), terpenoids, alkaloids (Chowdhary et al. 2013), phenolic compounds (flavonoids in particular) (El-Aziz et al. 2014), and volatile oils (Chowdhary et al. 2013). Furthermore, purslane shoots contain high levels of vitamins A, C, B1, B2, B3, B6, B9 (Azuka et al. 2014), and E (Kim et al. 2013), and minerals (Ca, Fe, Mg, Mn, K, Zn) (Azuka et al. 2014). This medicinal herb has bactericidal, antidiabetic, diuretic, anti-inflammatory, hepatoprotective, neuroprotective, anti-hyperlipidemic, anti-arthritic (Chowdhary et al. 2013) and anticancer effects (El-Aziz et al. 2014).

Antioxidant compounds are the first line of plant defense against free radicals. These compounds stabilize or deactivate free radicals before they can attack to the cells (Percival 1996). Phenolic compounds are the natural antioxidants including simple and complex phenolic compounds, flavonoids (such as anthocyanin, flavons, flavonols, and isoflavonoids) and tannins which occur widely in food plants (Özeker 1999).

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Omega-3 and omega-6 fatty acids belong to a group of PUFA containing 18 to 24 carbon atoms with three or more double bonds within the fatty acid chain (Uddin et al. 2014). In omega-3 fatty acids, the first double bond occurs on the third carbon atom, while omega-6, first double bond is 6 carbons from the methyl end of the fatty acid (Asif 2011). Omega-3 fatty acids are vital for normal growth and development of plants. Omega-3 fatty acids play an important role in prevention and treatment of diseases such as diabetes, cancer, arthritis, hypertension and inflammatory disorders (Simopoulos 2004).

Biochemical compositions of different plants are influenced by their phenological growth stages. For example, phenolic compounds and antioxidant activities of mature *P. oleracea* have been reported to be more than those at immature stages. Besides, the concentrations of Ca, Mg, K, Fe and Zn increase with plant maturity (Uddin et al. 2012). The amount of effective constituents of different plant species varies depending on the stage of growth (Toker 2009). For example, *Boerhavia diffusa* had maximum concentration of total phenols content at flowering stage (Verma and Kasera 2007), while in *Hypericum triquetrifolium*, the maximum amount of these compounds was determined at the full-flowering stage (Toker 2009). Furthermore, the highest antioxidant activity was observed at the full-flowering stage of *Satureja rechingeri* Jamzad plants (Alizadeh 2015). The concentration of chemical compounds in the same stages of development varies depending on the organs of the plant. For example, different organs of *Smilax campestris* had various fatty acid and phenolic contents. Flavonoid contents in its leaves were found to be more than the roots (Rugna et al. 2013). Furthermore, rosmarinic acid content was higher in the leaves of *P. vulgaris* compared to the stem, at all the examined phenological stages (Chen et al. 2012).

Purslane is an edible and medicinal plant with worldwide distribution. It was hypothesized that the concentration of its phytochemical compounds varies depending on the plant organs and phenological stages. Therefore, determining the best harvesting time and the organ in which the maximum quantity of desired metabolites is accumulated, contributes to the proper consumption of this plant. To the best of the authors' knowledge, no research about the effect of the phenological stage and organ type on the amount of phenolic, antioxidant and fatty acid content of purslane has been performed so far. In this regard, the phenolic compounds, fatty acids ( $\alpha$ -linolenic acid and linoleic acid) and antioxidant activity of *P. oleracea* in different growth stages and organs were evaluated. The results of the present study will address the knowledge gap about the appropriate harvesting of this medicinal plant.

## Materials and methods

### Plant materials

The seeds of purslane [Herbarium number 21808 (FUMH)] were collected from local herbs (Geographical coordinates: 34° 55' 17.94 " N, 59° 41' 2.11" E). Experiments were conducted in a greenhouse at faculty of agriculture, Ferdowsi University of Mashhad (Iran). The pots were arranged randomly at average greenhouse temperature (25/20 °C, day/night) and natural photoperiod (14/10 h, light and dark respectively). Seeds were germinated in the plastic pots (23 cm in diameter and 21.5 cm in height) filled with soil. The soil texture was loamy sand with reasonable nutrients content. During growing period, the pots were irrigated with water to maintain soil moisture around 0.8 field capacity (FC). The leaves and stems of plants were collected at 4 weeks (vegetative stage) and 8 weeks (flowering stage) after planting to analyze the fatty acid, phenolic contents and antioxidant activity. The samples (leaf and stem) were shadow-dried and finely powdered in a mechanical grinder. The experiment was conducted based on completely randomized design with three replications for all the treatments and phytochemical analyses.

### Preparation of purslane extracts

To measure phenolic compounds, flavonoids and antioxidant activities of the samples, the alcoholic extracts were prepared by soaking 0.25 g of the powdered plant in 25 mL of 80% aqueous methanol (v/v) for 24 h followed by filtration using Whatman filter paper No. 1. Then, the filtrates were evaporated under the fume hood (Fater Electronic, CH612, Iran). Afterwards, they were weighed and were stored in a freezer at  $-20$  °C.

### Total phenolic content

Total phenolic content of the extracts was determined using Folin–Ciocalteu reagent according to the procedure described by Singleton and Rossi (1965) with some modifications. For this purpose, 200  $\mu$ L of Folin–Ciocalteu reagent (10 times diluted), 500  $\mu$ L of water and 800  $\mu$ L of sodium carbonate (7%, w/v) solution were added to 1 mg mL<sup>-1</sup> of the methanolic extract. Finally, the absorbance was read after 30 min at 765 nm using the spectrometer (Shimadzu, UV-120-02, Japan). Using gallic acid (GAE) as the standard, total phenolic content was determined and expressed as mg GAE equivalents per 100 g dry weight.

### Total flavonoid content

In this test, 1 g of prepared extract was mixed with 1.5 mL of methanol, 0.1 mL of 10%  $\text{AlCl}_3$  (w/v in water), 0.1 mL of 1 M potassium acetate and 2.8 mL of water. After 30 min, the absorbance of the reaction mixture was measured at 415 nm. Calibration curve was generated with different concentrations of quercetin (QE) solutions and the results were expressed as mg QE equivalents per 100 g dry weight (Chang et al. 2002).

### Antioxidant activity

#### *DPPH free radical scavenging assay*

DPPH was assessed to determine the free radical scavenging activity of the extracts according to the method proposed by Brand-Williams et al. (1995) with minor modifications. Sample solutions with different concentrations (0.156–10 mg  $\text{mL}^{-1}$ ) were prepared from purslane methanolic extract. Then, 0.25 mL of each concentration was mixed with 1.75 mL of DPPH solution (0.025 mg  $\text{mL}^{-1}$ ). The mixture was left in darkness at room temperature for half an hour. The absorbance of the mixture was measured at 515 nm using the Shimadzu spectrometer. The equation below was used to compute DPPH% free radical scavenging of the samples:

$$\text{DPPH radical scavenging activity (\%)} \\ = \frac{[\text{Abscontrol} - \text{Absample}]}{\text{Abscontrol}} \times 100$$

#### *FRAP assay*

The FRAP assay was performed according to Sulaiman et al. (2011). For this purpose, FRAP reagent was prepared by mixing 10 mL of 300 mM acetate buffer at pH 3.6, 1 mL of 20 mM ferric chloride hexahydrate dissolved in distilled water and 1 mL of 10 mM 2,4,6-tri-(2-pyridyl)-s-triazine (TPTZ:  $\text{C}_3\text{N}_3[\text{C}_{15}\text{H}_{12}\text{N}_3]$ ) dissolved in 40 mM HCl. An aliquot (10  $\mu\text{L}$ ) of the extract (1 g  $\text{mL}^{-1}$ ) was added to 190  $\mu\text{L}$  of FRAP solution. After 30 min of mixture incubation at 37 °C, its absorbance was measured at 593 nm. Calibration curve was prepared with different concentrations of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  and the data were expressed in  $\mu\text{mol}$  of Fe per gram dry weight.

### Fatty acid content

For GC analysis, direct trans-esterification method was used based on the procedure reported by Lepage and Roy (1986) with some modifications. First, 1.5 mL of the extraction buffer containing methanol and 2% sulfuric acid (v/v) was added to 20 mg of the powdered purslane. Then,

the sample was incubated for 2 h at 80 °C in a shaker incubator (WiseCube, WIS-20R, Ukraine) at 750 rpm. Afterwards, 1.5 mL of 0.9% NaCl solution (w/v) and 1.5 mL of hexane were added to the reaction tube and the sample was centrifuged (3000 g, 20 °C, 3 min). The final supernatant was used for GC analysis (Matthew et al. 2009). The fatty acid (FA) was determined using a gas chromatography (6890, Agilent, USA) equipped with a flame ionization detector (FID). The oven was heated to 140 °C and kept there for 5 min, then raised to 240 °C at the rate of 4 °C  $\text{min}^{-1}$ . The temperature was programmed under the following conditions: carrier gas: Helium (1 mL  $\text{min}^{-1}$ ), split ratio: 20:1 and flame ionization detector (FID) temperature: 280 °C. Subsequently, retention time was compared with FAME standards to identify the fatty acid peaks. Standard samples of FA (including C18:2, and C18:3) were supplied by Supelco Company (USA, Catalog No: 18919-1AMP).

### Statistical analysis

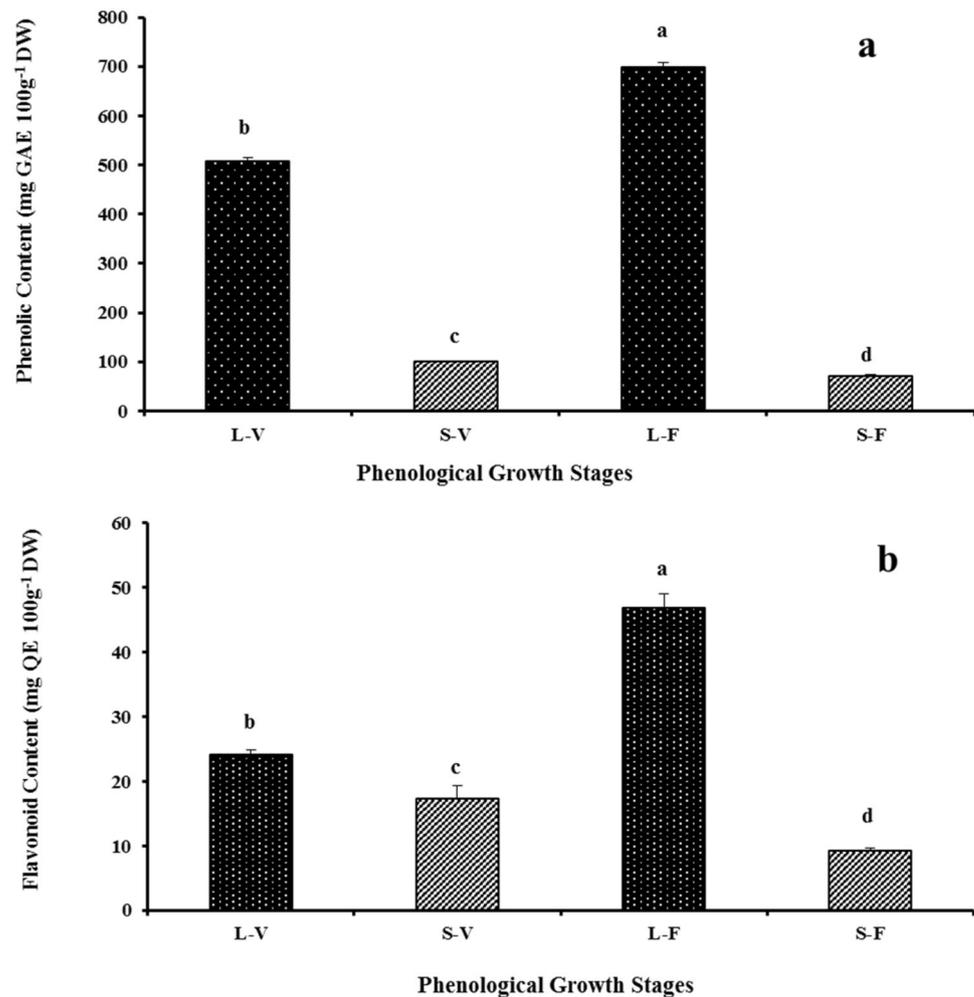
The data were analyzed using SPSS version 22 (SPSS, Inc., USA) and compared using one-way ANOVA, followed by Duncan test to evaluate significant differences among means with the confidence level ( $P \leq 0.05$ ). Pearson's correlation coefficient analyses used to assess the associations between different parameters.

## Results

### Total phenol compounds, flavonoid contents and antioxidant activity

The total phenol and flavonoid contents of the leaves and stems at different growth stages are shown in Fig. 1a, b, respectively. The maximum amount of total phenols was achieved in leaves at the flowering stage (698.6 mg GAE 100  $\text{g}^{-1}$  DW), and its minimum value was measured in the stems at this stage (70.9 mg GAE 100  $\text{g}^{-1}$  DW). As it can be seen, the content of total phenols was different between the plant organs. At the flowering stage, the total phenol content was 9.8 times higher in leaves compared to the stems. Besides, the total phenol content in leaves (506.6 mg GAE 100  $\text{g}^{-1}$  DW) was 5 times more than those in the stems (99.9 mg GAE 100  $\text{g}^{-1}$  DW). The amount of total phenols in the leaves at flowering stage (698.6 mg GAE 100  $\text{g}^{-1}$  DW) was reported to be 1.3 times higher than those that belonged to vegetative stage (506.6 mg GAE 100  $\text{g}^{-1}$  DW). However, levels of these compounds in the stems harvested at flowering stage (70.9 mg GAE 100  $\text{g}^{-1}$  DW) were less than the value of vegetative stage (99.9 mg GAE 100  $\text{g}^{-1}$  DW). Therefore, the maximum

**Fig. 1** Total phenols (a) and total flavonoid content (b) in leaf and stem of *Portulaca oleracea* at vegetative and flowering growth stages. Different small letters indicate significant difference between the means based on Duncan test ( $P \leq 0.05$ ). L-V (leaf-vegetation), S-V (stem-vegetation), L-F (leaf-flowering) and S-F (stem-flowering). Values are mean  $\pm$  S.D. of three replicates

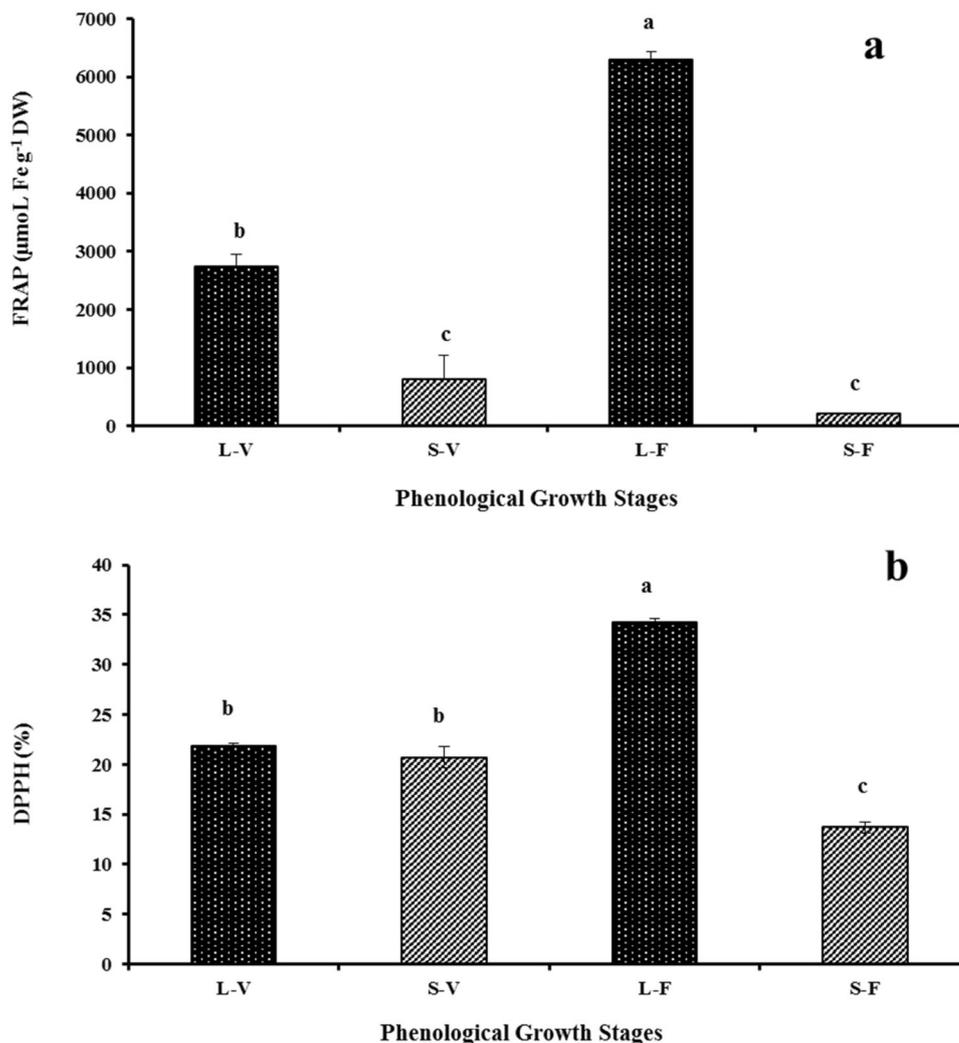


content of total phenol was obtained in the leaves, both in the vegetative and flowering stages.

Significant differences of the flavonoid content between leaf and stem were observed (Fig. 1b) ( $P \leq 0.05$ ). At the flowering stage, the flavonoid content for leaves (46.9 mg QE 100 g<sup>-1</sup> DW; the highest level of total flavonoid), was 5 times higher than the value measured in the stems (9.2 mg QE 100 g<sup>-1</sup> DW; the lowest level of total flavonoid). In the vegetative stage, the measured flavonoid levels in the leaves (24.2 mg QE 100 g<sup>-1</sup> DW) were 1.3 times higher than its content in the stems (17.3 mg QE 100 g<sup>-1</sup> DW). Changes in flavonoids content were affected by phenological stage. There was an increase of 1.9 times of flavonoids in the purslane leaves during flowering stage (46.9 mg QE 100 g<sup>-1</sup> DW) compared to vegetative stage (24.2 mg QE 100 g<sup>-1</sup> DW). Conversely, the flowering stage had a reverse effect on flavonoids content of the stems (9.2 mg QE 100 g<sup>-1</sup> DW) which was approximately half of its level measured at the vegetative stage (17.3 mg QE 100 g<sup>-1</sup> DW).

According to the results, the antioxidant activity of leaves (according to the FRAP method), significantly was greater than the stems ( $P \leq 0.05$ ), both in the vegetative and flowering stages (Fig. 2a). In the flowering stage, an increase of 30 times for FRAP was observed in the leaves (6305.3  $\mu\text{mol Fe g}^{-1}$  DW), when compared to stems (205.3  $\mu\text{mol Fe g}^{-1}$  DW). In the vegetative stage, the amount of FRAP measured in the leaves (2749.6  $\mu\text{mol Fe g}^{-1}$  DW) was about 3.4 times more than the amount estimated in stems (805.3  $\mu\text{mol Fe g}^{-1}$  DW). These results suggested a similar trend for antioxidant activity of leaves and stems during phenological stages. The amount of FRAP in the leaves, was significantly higher in the flowering stage than those at vegetative stage. Almost a similar trend was also recorded for the studied DPPH assay (Fig. 2b). Based on the results, antioxidant activity of leaves in flowering stage was significantly greater than the vegetative stage (approximately 1.5 times). Furthermore, a higher level of antioxidant activity was found in the leaves as compared with the stems at flowering stage. In this stage, the value of DPPH in the leaves (34.3%) was 2.5

**Fig. 2** FRAP (a) and DPPH free radical scavenging activity (b) data in leaf and stem of *Portulaca oleracea* at vegetative and flowering growth stages. Different small indicate significant difference between the means based on Duncan test ( $P \leq 0.05$ ). L-V (leaf-vegetation), S-V (stem-vegetation), L-F (leaf-flowering) and S-F (stem-flowering). Values are mean  $\pm$  S.D. of three replicates



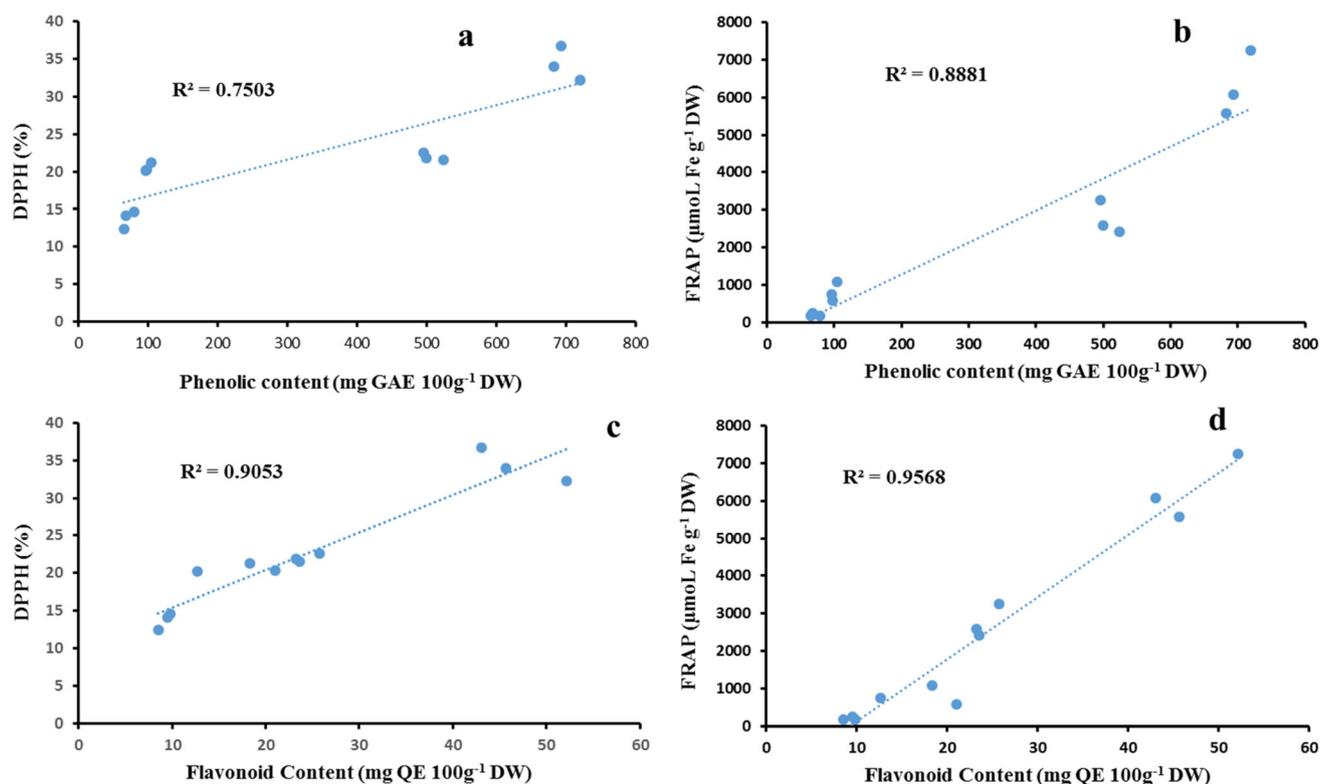
times more than that achieved in the stems (13.7%). No significant difference was measured between the organs at the vegetative stage.

Results of correlation analysis of the data demonstrated highly significant ( $P \leq 0.01$ ) positive correlation between the DPPH/FRAP values with total phenolic content ( $R^2$  for DPPH = 0.7503 and for FRAP = 0.8881), and with flavonoid content ( $R^2$  for DPPH = 0.9053 and for FRAP = 0.9568) (Fig. 3a–d). In this experiment, a strong and positive correlation ( $R^2 = 0.8731$ ) was found between the DPPH and FRAP values (Fig. 4).

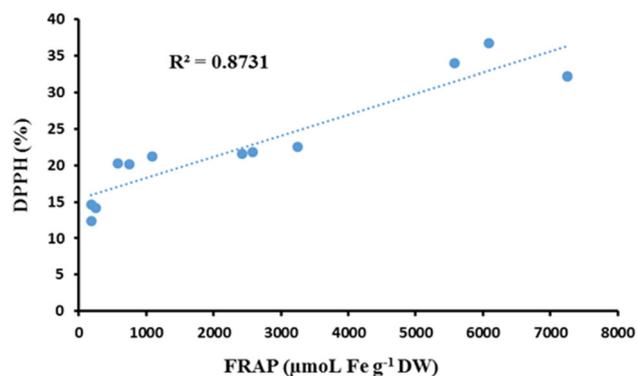
### Fatty acid contents

Fluctuations in fatty acids ( $\alpha$ -linolenic acid and linoleic acid) contents of purslane leaves and stems at vegetative and flowering growth stages are represented in Fig. 5a, b. The results showed that  $\alpha$ -linolenic acid and linoleic acid contents of leaves was significantly greater than the stems ( $P \leq 0.05$ ), both in the vegetative and flowering stages. At

the flowering stage, the amount of  $\alpha$ -linolenic acid in the leaves ( $2.7 \text{ mg g}^{-1} \text{ DW}$ ), was 6.7 times higher than that of measured in the stems at same time ( $0.4 \text{ mg g}^{-1} \text{ DW}$ ). In the vegetative stage, we found an increase of approximately 3 times in the amount of  $\alpha$ -linolenic acid in the leaves ( $1.4 \text{ mg g}^{-1} \text{ DW}$ ) compared to the stems ( $0.5 \text{ mg g}^{-1} \text{ DW}$ ). A similar trend was also observed for the studied linoleic acid. The highest and the lowest linolenic acid contents belonged to leaves at the flowering stage ( $0.8 \text{ mg g}^{-1} \text{ DW}$ ), and the stems at the vegetative stage ( $0.3 \text{ mg g}^{-1} \text{ DW}$ ) respectively. At flowering stage, the content of linoleic acid in the leaves ( $0.8 \text{ mg g}^{-1} \text{ DW}$ ) was enhanced 2 times more than the stems at the same time ( $0.4 \text{ mg g}^{-1} \text{ DW}$ ). In this way, an increase of 1.6 times was found in the amount of linoleic acid in the leaves ( $0.5 \text{ mg g}^{-1} \text{ DW}$ ) compared to stems ( $0.3 \text{ mg g}^{-1} \text{ DW}$ ) at the vegetative stage. In general, phenological stage had significant effect on the accumulation of fatty acids in the leaves but not in the stems. An increase of 1.9 times in the amount of  $\alpha$ -linolenic acid in the leaves at the flowering



**Fig. 3** The correlation between phenolic content and antioxidant activity (DPPH) (a), and FRAP (b) as well as between flavonoid content and antioxidant activity (DPPH%) (c), and FRAP (d)



**Fig. 4** The correlation between antioxidant activity (DPPH and FRAP)

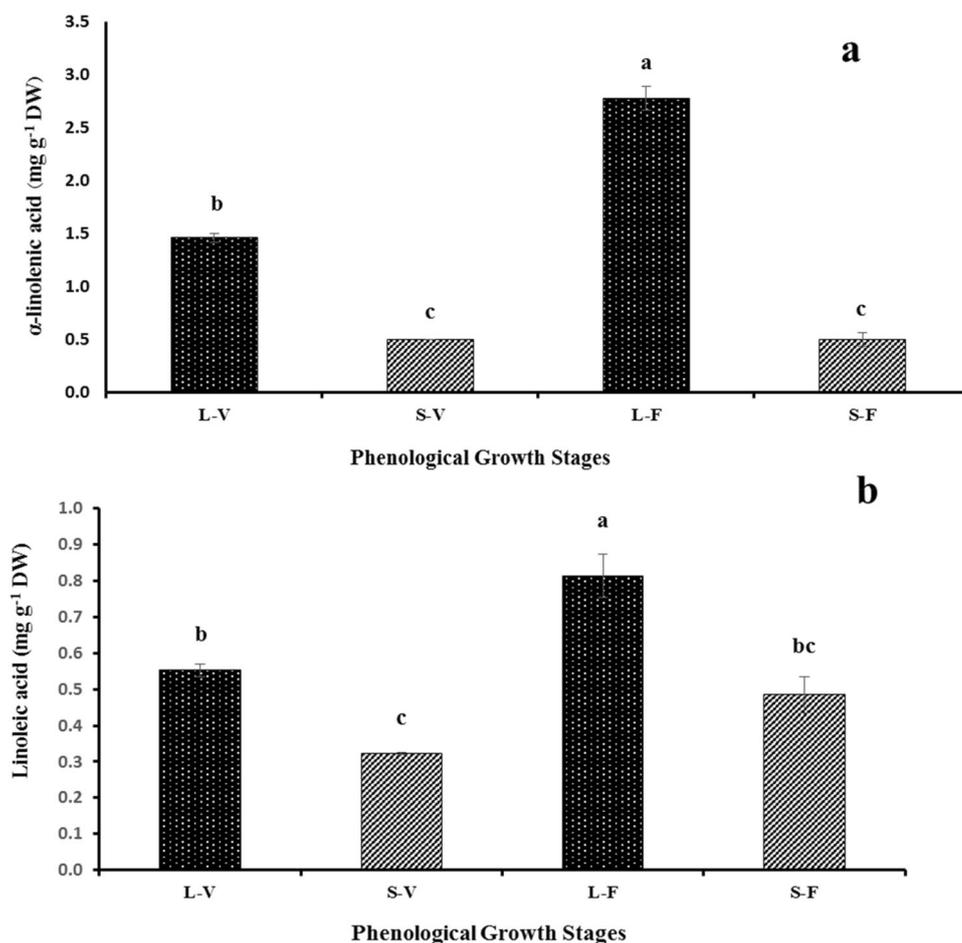
stage (2.7 mg g<sup>-1</sup> DW) was observed compared to the vegetative stage (1.4 mg g<sup>-1</sup> DW). For linoleic acid, 1.6 fold increases was observed in the leaves at the flowering stage (0.8 mg g<sup>-1</sup> DW) compared to the vegetative stage (0.5 mg g<sup>-1</sup> DW).

In this experiment, a positive correlation was found between DPPH/FRAP values with linoleic acid content (R<sup>2</sup> for DPPH = 0.5240 and for FRAP = 0.6804) and with  $\alpha$ -linolenic content (R<sup>2</sup> for DPPH = 0.8343 and for FRAP = 0.9313) (Fig. 6).

## Discussion

Fluctuation in the amounts of phenolic compounds can be attributed to several factors including intrinsic factors such as the plant species, organ and age of plants and extrinsic factors like biotic and abiotic factors. Our study demonstrated that the total phenol content in *P. oleracea* varied from 70.9 to 698.6 mg GAE 100 g<sup>-1</sup> which was consistent with the results of Uddin et al. (2012) about *P. oleracea*. Our results showed that the amount of phenolic compounds in the leaves significantly was greater than the stems, both in flowering and vegetative stages (Fig. 1a). In flowering stage, the phenolic content of leaves (698.6 mg GAE 100 g<sup>-1</sup> DW), was 9.8 times higher than the stems at the same time (70.9 mg GAE 100 g<sup>-1</sup> DW). According to the results of Petropoulos et al. (2019), oleracein C content in purslane leaves was reported more than the stems at all the harvesting stages (29, 43, and 52 days after sowing). According to our results, 1.3 times increase in the phenolic content of the leaves was observed in the flowering stage compared to vegetative stage (Fig. 1a). Many studies have demonstrated that vegetable plants have a peak level of phenolic compounds in the reproductive growth stage (Sellami et al. 2009; Fernando et al. 2013). Verma and Kasera (2007) also reported that the flowering stage of *B.*

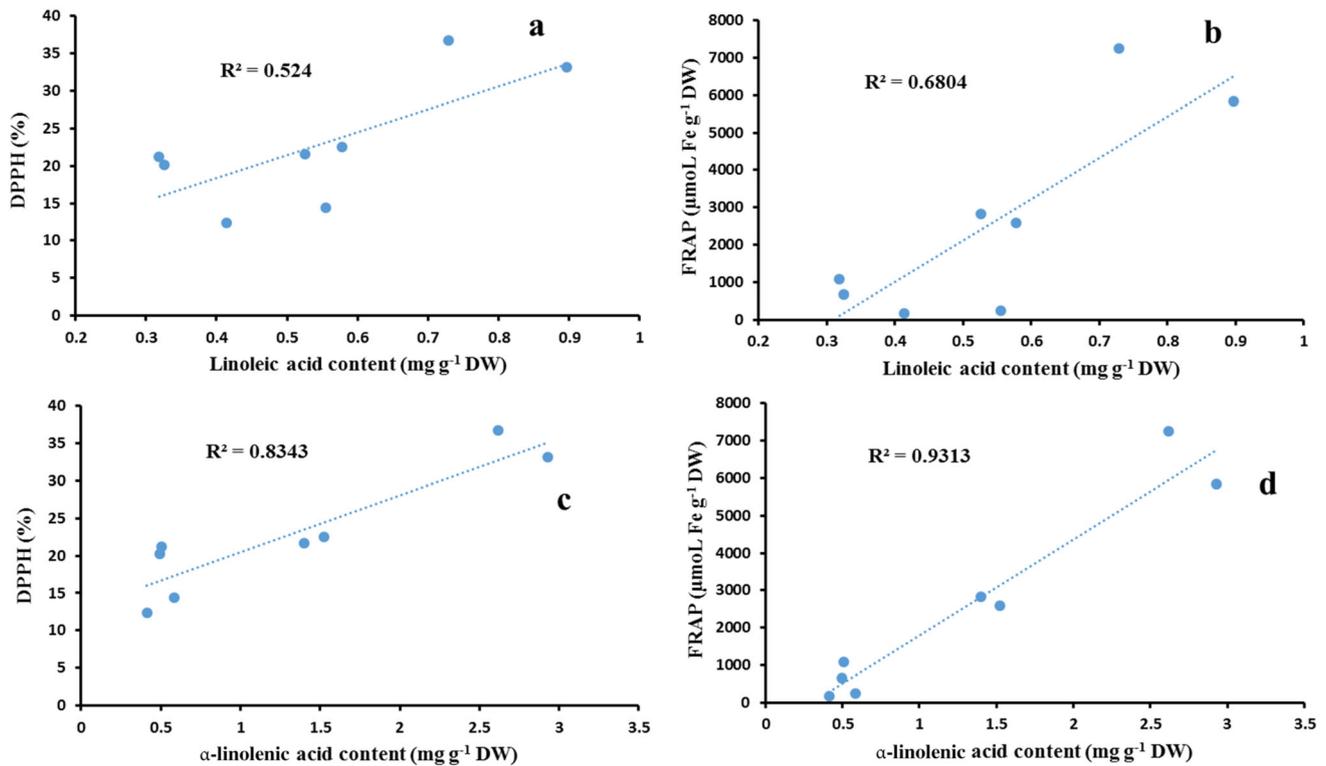
**Fig. 5**  $\alpha$ -linolenic acid (a) and linoleic acid contents (b) in leaf and stem of *Portulaca oleracea* at vegetative and flowering growth stages. Different small letters indicate significant difference between the means based on Duncan test ( $P \leq 0.05$ ). *L-V* (leaf-vegetation), *S-V* (stem-vegetation), *L-F* (leaf-flowering) and *S-F* (stem-flowering). Values are mean  $\pm$  S.D. of three replicates



*diffusa* and *Sida cordifolia* had the maximum concentration of total phenolics, but there were opposite results in *Asparagus racemosus*. The highest total phenolic and flavonoid contents of *Hypericum triquetrifolium* shoot was found at full flowering stage (Toker 2009). An increase in the activity of phenylalanine ammonia lyase enzyme (PAL) in reproductive phase may be the main reason for enhancing phenolic compounds in the flowering stage (Andreotti et al. 2006). Some multifunctional transcription factors and regulator proteins have been proposed to be responsible for the development-dependent accumulation of phenolic compounds in the plant tissues (Jiang et al. 2013).

Significant differences ( $P \leq 0.05$ ) were observed among leaves and stems with regard to flavonoid content (Fig. 1b). The flavonoid content at flowering stage in leaves (46.9 mg QE 100 g<sup>-1</sup> DW) was 5 times more than the stem at this stage (9.2 mg QE 100 g<sup>-1</sup> DW). These results are in line with the numerous literature data reporting high levels of these compounds in the leaves. Veit et al. (1995) reported the tissue-specific fluctuation of various flavonoids and caffeic acid esters in different organs of *Equisetum* species. Phenological stage had a significant effect on total

flavonoid content (Fig. 1b). There was an increase of 1.9 times of flavonoids in the purslane leaves during flowering stage (46.9 mg QE 100 g<sup>-1</sup> DW) compared to vegetative stage (24.2 mg QE 100 g<sup>-1</sup> DW). Similarly, in another survey on tabat Barito (*Ficus deltoidea* Jack), the highest and lowest flavonoid content was found in senescent leaves and stem, respectively (Manurung et al. 2017). In this way, a close relationship can be proposed during the processes of biosynthesis, degradation and transport in the distribution of phenolic compounds throughout the plant (del Baño et al. 2003). Phenolic acid and flavonol contents in aerial parts of plants are usually higher than roots. To identify putative genes involved in phenolic compounds biosynthesis, several genes have been investigated. Expression pattern of these genes and their multiple isoforms varied by organs and growth stages. In an experiment on *Sophora flavescens*, the expression pattern of the genes encoding enzymes involved in the phenylpropanoid pathway and some flavonoid pathway-specific enzyme isoforms were affected by the organs or phenological stage. In this experiment, *SfPAL*, the gene encoding the first enzyme in the phenylpropanoid biosynthetic pathway, was expressed at high levels in the medium leaves, but expressed at low



**Fig. 6** The correlation between linoleic acid content and antioxidant activity (DPPH) (a), and FRAP (b) as well as between  $\alpha$ -linolenic acid content and antioxidant activity (DPPH%) (c), and FRAP (d)

levels in other organs. Varied expression depending on organ or phenological stage was also seen for genes encoding the central enzymes of phenylpropanoid pathway (*SfC4H* and *Sf4CL*). The transcript *SfC4H-1* was expressed in all vegetative organ; it decreased as leaves grew and increased as stems grew. The other transcript (*SfC4H-2*), expressed at the highest level in small stem. Also in this study, the expression patterns of three *SfCHS* isoforms, the first enzyme specific for the flavonoid pathway, were affected by organs and phenological stages. In general, researchers of this experiment believe that several genes or their multiple transcript isoforms that are involved in the biosynthesis of various phenolic compounds in *Sophora flavescens*, have spatially and temporally specific expression patterns (Lee et al. 2018). The activity of PAL as a key enzyme of the phenylpropanoid pathway was enhanced with increasing the production of phenylpropanoids products, and varied with the phenological stages (Bagal et al. 2012). Different expression patterns of various copies of genes encoding the phenylpropanoid pathway enzymes (PAL, and cinnamate-4-hydroxylase) are related to physiological conditions and development. This might be a reason for the variable production of different phenolic compounds (Bagal et al. 2012; Xia et al. 2017).

Our results showed that the FRAP/DPPH values in leaves were significantly greater than the stems, both at

flowering and vegetative stages (Fig. 2a, b). FRAP value in leaves harvested at the flowering stage was 30 times more than that of the stems at this stage. In the stems, FRAP value did not vary considerably during phenological stages. Similar results are obtained by Oliveira et al. (2009). The phenological stage, like the organ type, had a significant effect on FRAP and DPPH values (Fig. 2b). Alizadeh (2015) found that the *Satureja rechingeri* extract derived at the full-flowering stage was most effective in DPPH radical scavenging and showed the highest FRAP values. Manurung et al. (2017) revealed that nascent leaves had higher antioxidant activity compared to young leaves and stem. It should be noted that numerous synthesis and degradation processes of antioxidant compounds may take place during maturation, leading to changes in the antioxidant capacity of the plants. During the physiological process of plant ontogenesis, morphological modifications in plants are concomitant with modifications in the secondary metabolisms. Therefore, the variations in phenolic compounds and antioxidant activity during phenological stages may be closely related to the metabolic and physiological changes throughout the plant life cycle (Chouaieb et al. 2012).

In this experiment, a high and positive correlation was observed between antioxidant activity and the contents of phenolic and flavonoid compounds (Fig. 3). These results

are consistent with studies on purslane (Nagarani et al. 2014; Alam et al. 2015). The potential antioxidant activity of purslane leaves as well as its correlation with the phenolic compounds was demonstrated by Alam et al. (2015) and Nagarani et al. (2014). Total phenolic and flavonoid contents were reported to be associated with antioxidant potentials in plants (Uddin et al. 2012; Chouaieb et al. 2012). In this way, Chirinos et al. (2007) showed a significant correlation between antioxidant capacity and total phenolic content in *Tropaeolum tuberosum*. Phenolic compounds are major endogenous antioxidants in plant cells. Because the phenolic compounds are the antioxidants of the intrinsic cells of the plants, the relationship between the antioxidant capacity and the amount of these compounds seems reasonable (Zhao 2015). Since the formations of reactive oxygen species could be neutralized by phenolic compounds (e.g. flavonoids), the enhancement in their content results in increasing the antioxidant capacities (Noriham et al. 2015).

The  $\alpha$ -linolenic acid content of purslane was measured as 0.4–2.7 mg g<sup>-1</sup> DW which is consistent with the results of Siriamornpun and Suttajit (2010) at *P. oleracea*. The amount of  $\alpha$ -linolenic acid and linoleic acid in leaves, was significantly greater than the stems, both in the vegetative and flowering stages (Fig. 5a, b). So that the amount of  $\alpha$ -linolenic acid in leaves at flowering stage (2.7 mg g<sup>-1</sup> DW) was 6.7 times higher than stem at same time (0.4 mg g<sup>-1</sup> DW). Linoleic acid content reported in leaves (0.8 mg g<sup>-1</sup> DW) was about 2 times higher than the stem (0.4 mg g<sup>-1</sup> DW) at flowering and vegetative stage. The investigation of the level of unsaturated fatty acids in different organs of some Iranian *Echium* revealed higher amounts of these compounds in the leaves rather than the stems (Abbaszadeh et al. 2011). In agreement with the our results, Uddin et al. (2014) showed higher fatty acid contents in the leaves compared to the stems. In the present study, the phenological stage had a significant effect on the amount of fatty acids in the leaves (Fig. 5a, b). An increase of 1.9 times for  $\alpha$ -linolenic acid and 1.6 times for linoleic acid observed in leaves at flowering stage compared to vegetative stage. As Peiretti and Gai (2009) concluded, linoleic acid content showed an increase with getting further in growth stage of chia plant (*Salvia hispanica* L.). The absorption of a wide range of metabolites by plastid for fatty acid synthesis depends on the plant species, organ and development stage (Rawsthorne 2002). In general, an enhancement of the fatty acids amount during growth may be due to increased production of total lipid or examined fatty acids. Phenological stage may affect the overall enzymes involved in lipid metabolism, such as Acetyl-CoA carboxylase (ACCase), and/or 3-ketoacyl-ACP synthase (KAS) increasing total lipid. ACCase is an important regulatory enzyme in the pathway of fatty acids biosynthesis

that catalyzes the first stage of carboxylation of acetyl-CoA to malonyl-CoA. Another important enzyme of this pathway is KAS playing as a condensing enzyme (Murphy 2009). Probably, these genes are differentially expressed at different phenological stages (Gu et al. 2012). It is also suggested that the activity and/or expression level of specific enzymes involved in omega-3 and/or omega-6 biosynthesis may be regulated by different factors such as phenological stage. A correlation was observed between the growth rate of a cell population and the unsaturation level of 18C fatty acids by Mei et al. (2015). Studies have shown that fatty acid desaturases including FAD2 and FAD3 (responsible for the formation of 18:2 and 18:3 on phospholipids, respectively) have limiting activities in fast-growing cultures. It might be possible that in situations where membrane syntheses and/or turnover are fast, the activities of FAD2/FAD3 enzymes are not sufficient to match the phospholipid synthesis (Mei et al. 2015).

Pearson's correlation coefficient analysis showed that there was a high and positive correlations between amounts of PUFAs ( $\alpha$ -linolenic acid and linoleic acid) and antioxidant activity (Fig. 6). Consistent with the present study, a positive correlation was found between the antioxidant activity (DPPH values) of leguminous seeds (Grela et al. 2017), quinoa seeds (*Chenopodium quinoa* Willd.) (Tang et al. 2015) and avocado (*Persea americana* Mill.) with PUFA contents (Villa-Rodríguez et al. 2011). Previous studies have shown that PUFAs, especially omega-3, play a role in inhibiting superoxide (Grela et al. 2017).

## Conclusion

As a conclusion, phenological growth stages and plant organs significantly influenced the antioxidant activity, total phenolics and  $\alpha$ -linolenic acid and linoleic acid contents. In this study, through all the phenological stages, the mature leaves of *P. oleracea* exhibited the highest total phenolic and fatty acid ( $\alpha$ -linolenic acid and linoleic acid) contents, as well as antioxidant activities compared to the younger leaves and stem. Thus, phenological growth stage can significantly affect the quality of purslane leaves and their potential antioxidant activity. Regarding the medicinal properties of this edible plant, the mature leaves of purslane (at flowering stage) can serve as a good source of fatty acids ( $\alpha$ -linolenic acid and linoleic acid) and phenolic compounds with proper antioxidant activities.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

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