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Foliar application of 5-aminolevulinic acid promotes bioactive compounds and nutritional value of purslane, a potential vegetable for the future

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Summary

Decent taste and the salt and drought tolerance of purslane (*Portulaca oleracea* L.) make it a potential vegetable crop for the future. This study investigates the effects of foliar application of 5-aminolevulinic acid (5-ALA), a new plant growth regulator, on the growth, mineral composition, and bioactive compounds of purslane. Three different levels of 5-ALA (0, 25 and 50 mg L^{-1}) were sprayed at 1) two leaved stage and 2) upon the onset of inflorescence appearance on purslane seedlings. Results showed that 5-ALA application enhanced biomass accumulation in the plant shoot and increased shoot length. Concentration of nitrogen, potassium, magnesium, zinc and iron increased in the shoots of 5-ALA treated plants, while the calcium concentration remained unaffected. Phenolic compounds of the plant were catechin, chlorogenic acid, and ellagic acid, with catechin being the main compound. Further on, Trans-ferulic acid, hesperedin and eugenol were detected in the extract of 5-ALA-treated plants. Application of 5-ALA also increased fatty acids in the plant leaves. Total phenolics, ascorbic acid contents and antioxidative activity of shoot were increased in the 5-ALA-treated plants. Moreover, pH of root exudates of the plants was decreased in 5-ALA treated plants. The results revealed that exogenous 5-ALA has growth regulatory effects and can enhance the growth, and improve nutritional quality and pharmaceutical properties of *P. oleracea*. In this regard, the best results were obtained by application of 50 mg 5-ALA L^{-1}.

Keywords: fatty acids, mineral nutrients, organic acids, purslane, phenolic compounds, root exudation.

Fig. 1: Purslane is a hardy species with capability to grow in hot and dry environmental conditions. This picture indicates how the plant can survive abiotic challenges such as drought and poor soil. The fleshy nutritious leaves of the plant have decent salty/sour taste. These specifications make it a potential vegetable crop for the future.

Introduction

Dietary fiber, polyunsaturated fatty acids, and antioxidantive phenols, flavonoids, terpenoids, and vitamins of vegetables are of critical importance for consumer health (Barros et al., 2008). Purslane (*Portulaca oleracea* L.) is a tolerant species with capability of growing in nutrient-poor soils and harsh environmental conditions such as drought and salt affected soils. The plant is well adopted to hot and dry conditions and can survive under new climate of the globe (Fig. 1). In addition to gentle salty/sour taste, it has high nutritious value i.e. high content of linoleic (18:2ω6) and α-linolenic (18:3ω3) acids, and antioxidative compounds (Mera-Ovando et al., 2014; Siriamornpun and Suttajit, 2010). Based on the USDA Nutrient Database (2018), 100 gr of raw purslane may provide 81% vitamin E, 25% vitamin C, 19% magnesium, 15% iron and 11% potassium of daily requirements for the adults. In sum, these characteristics make purslane a potential food resource for future. The plant is an individual from *Portulacaceae* which contains many species whose number exceeds 120 that are succulent herbs and bushes (Hyam and Pankhurst, 1995). The aerial organs of these plants are used as a febrifuge, antiseptic, diuretic, vermifuge and antispasmodic (Xiang et al., 2005). The plant has antidiabetic effects (Sharma et al., 2010) and can be used for reducing triglycerides and cholesterol in blood (Zidan et al., 2014). Furthermore, antiviral (Cai-Xia et al., 2010) and antitumoral (Zhao et al., 2013) have been noticed in the plant extract. In some regions, especially in West Asia, purslane is consumed as a foliar vegetable, too. It is known to have advanced nutritional value than many cultivated vegetables, as a boundless source of ascorbic acid, phenolics and antioxidants (Liu et al., 2000).

The nutraceutical and bioactive compounds of plant crops can be improved by optimizing growing conditions, cultivation practices and application of biostimulators. In this regard, plant growth regulators are an excellent choice for manipulating plant metabolites and pharmacological effects. 5-aminolevulinic acid (5-ALA) is a plant growth regulator (PGR) which can enhance different types of antioxidants in plant tissues (Nishihara et al., 2003). It is a precursor of tetrapyrrole compounds such as heme, chlorophylls, and cobalamin (Stobart and Ameen-Bukhari, 1984). In bacteria and plants, prosthetic groups of respiratory enzymes and chlorophylls are provided by this compound (Brunham and Lascelles, 1963). Application of low concentrations of 5-ALA (10-300 mg L^{-1}) at early growth stage, have been reported to increase yield of barley, potato, radish, garlic and kidney bean by enhancing growth rates and photosynthesis of these species (Hotta

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et al., 1997). WATANABE et al. (2006) reported that application of 100 mg L^{-1} of 5-ALA increased the growth and CO_2 assimilation of grapevine. 5-ALA at 10 and 100 mg L^{-1} enhanced chlorophyll biosynthesis and photosynthesis rate in melon seedlings under low light intensity (WANG et al., 2004).

This chemical is effective at low concentrations (76.2-762 µM) and seems to be a new PGR. The ameliorative effect of 5-ALA on accumulation of simple sugars accompanied by the maintenance of starch content in the leaves and preserving relatively higher leaf mineral contents in different plant species such as rapeseed (LIU et al., 2016) and sunflower (AKRAM and ASHRAF, 2011) have been demonstrated previously. The enhancement of the cell antioxidants is another effect of 5-ALA which provides significant protection to plasma membranes against free reactive oxygen radicals (NISHIHARA et al., 2003). Therefore, it can be used as a PGR to manipulate secondary metabolites and promote pharmaceutical properties of medicinal plants. However, there is certainly lack of comprehensive understanding of physiological and biochemical responses of medicinal plants to 5-ALA. According to the role of 5-ALA on the accumulation of secondary metabolites and bioactive compounds, this study was to evaluate growth, phenolics, antioxidative activity and mineral composition of *P. oleracea* L. in response to foliar application of 5-ALA, in an effort to improve its application for the public consumption.

Material and methods

Plant material and growing condition

A greenhouse experiment was completed at the Shiraz Payame Noor University. The soil used in this study was a fine topsoil loam taken from 0 to 30 cm of a calcareous soil (Typic Calcixerepts). Physicochemical properties of the soil were as follows: pH: 7.41, EC_e: 1.2 dS m^{-1}, CEC: 10 $cmol_c$ kg^{-1}, organic carbon: 8.9 g kg^{-1} soil, nitrogen (N): 0.07%, phosphorus (P): 13 mg kg^{-1} soil, potassium (K): 59 mg kg^{-1} soil, calcium carbonate equivalent (CCE): 454 mg kg^{-1} soil, DTPA-extractable Fe: 2.21 mg kg $soil^{-1}$. Nitrogen and phosphorous at the concentration of 50 mg kg^{-1} soil, and Cu, Zn and Mn at the concentration of 5 mg kg^{-1} soil were applied in a uniform manner to the soil each as NH_4NO_3, KH_2PO_4, $CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$ and $MnSO_4 \cdot H_2O$, respectively. 8-liter plastic pots were filled with 7.5 kg of the soil.

Twenty seeds of purslane (*Portulaca oleracea* L.) were planted in pots and irrigated with deionized water to field capacity twice a week. After fifteen days, at two-leaf stage, the seedlings were thinned to 10 uniform stands in each pot. Three concentrations of 5-ALA (0, 25 and 50 mg L^{-1} in the form of δ-Aminolevulinic acid hydrochloride) was sprayed on the plants at two stages: after thinning (20 days after planting) and upon the onset of inflorescence appearance (65 days after planting). Deionized water was also sprayed as control. The plants were harvested 12 weeks after planting. Shoot fresh (FM) and dry mass (DM), and shoot length were measured after twelve weeks. Moreover, the following physiological and biochemical traits were analyzed.

Purslane extracts preparation

Methanol extracts of *P. oleracae* L. samples were prepared by the method described in NAJAFIAN and ZAHEDIFAR (2015). Twenty grams of dried samples were drenched in 250 mL of methanol/water (90:10 v/v) for 48 hours. Filtration and concentration of the extracts were done in a rotary evaporator for ten minutes. The desiccated extracts were powdered and by measuring the mass of the fine powders their yields were determined. The powders were maintained at -18 °C before utilization. The needed level of powder in methanol was arranged before measurements, and then total phenol content and the antioxidative activity were evaluated.

Extraction and HPLC analysis of polyphenols

Extraction of polyphenols was carried out in view of a previous report with a few modifications (JUSTESEN et al., 1998). HPLC examination was performed on an Agilent Technologies 1200 series (Germany), outfitted with a Zorbax eclipse (XDB) C_{18} (5 µm (ID), 4.6 × 150 mm (FT) and a photodiode cluster identifier. At 230 and 280 nm, elution was observed. Elution was performed by changing the solvent methanol to formic acid proportion.

Total phenolics and ascorbic acid contents

The total phenolic compounds (TP) in the plant extracts were measured by using the Folin-Ciocalteu reagent. According to the method described by HALICIA et al. (2005), 1.2 mL of Na_2CO_3 (7.5%, w/v) solution and 1.5 mL of the reagent (diluted 10 times) were added to 300 mL of samples. The mixtures were shaken and left at a dark room for 30 minutes. Then the mixture absorbance was measured at 765 nm by a spectrophotometer (Varian 220, Australia). The measurements were repeated 3 times. The total phenolics' content was represented as gallic acid (GAE) equivalent in 100 g fresh sample. Amendments TP content was made by subtracting the ascorbic acid content (AA) from the total phenolics content.

Ascorbic acid content in the plant leaves was measured using indophenol method according to AOAC (1984). 10 g of fresh leaves were homogenized with 48 ml metaphosphoric acetic acid and 2 ml of sodium citrate solution. The samples were filtered using a Buchner funnel and suction pump and 10 milliliters of the filtrates were rapidly titrated with the standardized 2,6-dichlorophenolindophenol solution till formation of a permanent pink color. The titrations were repeated 3 times. Blank determinations were also carried out by using 10 ml of metaphosphoric acetic acid instead of the filtrate.

Antioxidative activity measurement

For measuring the scavenging capacity of the plant extracts against DPPH free radical, the 25 µL of 12-3100 µg mL^{-1} gallic acid or methanol extracts was blended with 220 µL of 120 mM of DPPH in methanol (BURITS et al., 2001). For thirty minutes, the solutions were retained at ambient temperature. DPPH radical restraint was assessed at 515 nm utilizing an EL×808 absorbance microplate reader (BioTek Instruments Inc., USA). Concentration (mg L^{-1}) needed to restrain DPPH radical formation by fifty percent (IC_{50}) were computed from the nonlinear regression between mean % radical-scavenging activity utilizing MATLAB software (The MathWorks Inc., USA) and log extract concentration (g mL^{-1}). Methanol extract without DPPH used as the blank. Antioxidant activity was assigned utilizing the following equation:

$$\text{Antioxidant activity} = 1 - [(A_{sample} - A_{blank})/A_{control}] \times 100$$

Where A_{sample} and A_{blank} are absorption of the test solution (t = 30 min) and the blank reaction (t = 0 min). Control and blank solutions were DPPH (without plant extract) and methanol solutions, respectively.

Fatty acids content

Fatty acids were determined according to the method described by BARROS et al. (2008). The fatty acids were methylated with methanol:sulphuric acid:toluene 2:1:1 (v:v), during 12 h at 50 °C. Deionized water was added to the solution to obtain phase separation. After vortexing, the fatty acids methyl ester were recovered with diethyl ether. The upper phase was passed through a micro-column of sodium sulphate anhydrous and then filter by a 0.2 µm nylon filter. The fatty acid profile was analyzed using a GC-MS (Agilent 7890 system, Agilent Technologies Inc., USA). The instrument equipped with a split/splitless injector, a flame ionization detector (FID) and a Macherey-Nagel column (30 m * 0.32 mm ID * 0.25 µm df). The

initial temperature of the column was 50 °C, held for 2 min, then a 10 °C/ min ramp to 240 °C and held for 11 min. Flow rate of the carrier gas (hydrogen) at 50 °C was 4.0 mL/min (0.61 bar). Split injection (1:40) was carried out at 250 °C. One µL of the sample was injected and fatty acid identification was made by comparing the relative retention times from samples with FAME peaks (standards). The results were recorded and processed using CSW 1.7 software (DataApex 1.7) and expressed in relative percentage of each fatty acid.

Root exudates analysis

The plants' roots were carefully washed off the soil and used for analyzing organic acids in their exudates according to the method described by CZARNOTA et al. (2001). Roots were extracted with methylene chloride acidified with 0.25 % glacial acetic acid, for 30 s. The extract was filtered with a 2.0 µm filter and freeze dried. The concentration of organic acids was determined in freeze dried root exudates. The samples were dissolved in with ethanol (80%). Then, 20 microliter of the dissolved extracts were injected into Zorbax eclipse (XDB) C18, 4.6 (ID) × 150 mm, 5 µm (film thickness) column. The column temperature was 45 °C. Agilent HPLC 1200 series was used to determine concentration of organic acids in the root exudates. The mobile phase was a mixture of acetonitrile: sulfuric acid: acetic acid (15:4:1) with the flow rate of 1 ml min^{-1} for 10 minutes. The photodiode array detector was set on 214 nm for quantification of organic acids. For measurement of pH, freeze-dried samples were dissolved in deionized water and pH of the extracts was measured using a pH meter (HANNA Model HI2210, USA).

Mineral composition of plant

Shoot samples were dried for 72 h at 70 °C in an oven. Nitrogen concentration of the samples was determined by Autotech (Model 300). Then samples (ca. one gram per replicate) were ashed for 6 h at 550 °C. The white ash powder was added in 5 mL of 2 M hot hydrochloric acid, filtered into a 50 mL volumetric flask, and made up to 50 mL with deionized water. Iron, calcium, magnesium, and zinc analyses were done using an atomic absorption spectrophotometer (Model Varian 220, Australia). By using a flame photometer, the concentration of potassium in the plant extracts was determined.

Statistical analysis

The experimental design was a completely randomized design with three treatments. Eight replications were considered per treatment and ten seedlings used per replicate. In sum, 240 plants were used in this experiment. Statistical analysis was carried out by the statistical software SPSS (version 20). Duncan's multiple range test (DMRT) at the level of 5% probability was utilized to compare the differences between the means.

Results

Plant growth

Tab. 1 represents the effects of 5-ALA on plant growth indices over twelve weeks. The shoot dry mass, fresh mass and mean of shoots' length of purslane seedlings showed an increasing trend with application of 5-ALA. The highest shoot growth parameters of the plants were obtained with application of 50 mg L^{-1} 5-ALA.

Total phenolics and ascorbic acid contents

Compared to the control, 5-ALA applications increased the total polyphenolics (TP) and ascorbic acid (AA) contents in the aerial organs of purslane (Fig. 2A, B). No significant differences were observed in TP and AA levels in the leaves of 25 or 50 mg L^{-1} 5-ALA treated plants. The highest total phenolics (3.67 g GAE 100 g^{-1}) and ascorbic acid content (81.47 mg 100 g^{-1}) was found in plants supplied with 25 mg L^{-1} 5-ALA. TP content was positively associated with the antioxidative activity and ascorbic acid content in the plant leaves (Fig. 3A, B).

Tab. 1: Shoot fresh mass (FM) and dry mass (DM) and mean of shoots' length of *Portulaca oleracea* in response to foliar application of different 5-ALA concentrations.

Measurements	5-ALA (mg L^{-1})		
	0	25	50
FM (g)	$34.53^{c} \pm 0.73$	$49.97^{b} \pm 1.00$	$57.64^{a} \pm 1.41$
DM (g)	$2.42^{c} \pm 0.32$	$3.19^{b} \pm 0.31$	$6.98^{a} \pm 0.21$
Shoot length (cm)	$19.44^{b} \pm 1.64$	$21.45^{b} \pm 1.75$	$34.26^{a} \pm 1.73$

*Means followed by the same letter are not significantly different according to DMRT at $P \leq 0.05$. Data are mean ±standard deviation of eight replications.

Phenolic compounds

In this study, 3 phenolic compounds (catechin, chlorogenic acid, and ellagic acid) were detected in the control plants (Tab. 2). With the exception of chlorogenic acid, 5-ALA application had outstandingly positive effects on concentration of these phenolic compounds. In this regard, foliar application of 50 mg L^{-1} 5-ALA incited significant higher phenolics in comparison with the 25 mg L^{-1} treated plants. Catechin was the predominant phenolic compound in the control

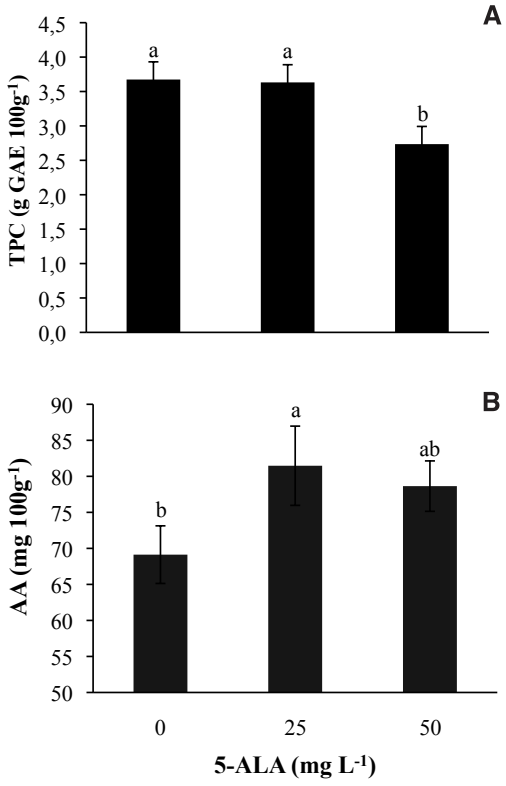

Fig. 2: The effects of different 5-ALA treatments on total phenolics (TP) (A) and ascorbic acid (AA) contents (B) in the leaves of *Portulaca oleracea*. Means marked by the same letter are not significantly different according to DMRT at $P \leq 0.05$. Error bars represent standard deviation.

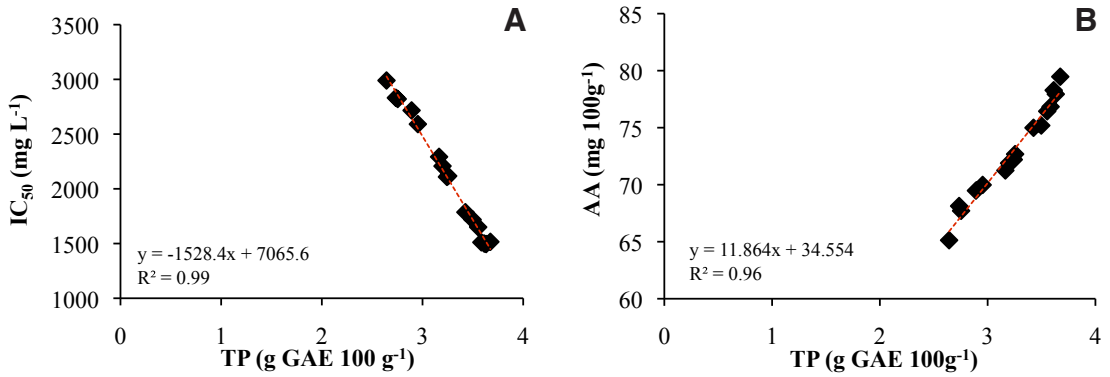

Fig. 3: Correlations between (A) total phenolics (TP) content and antioxidant activity (IC_{50}) and (B) between total phenolics and ascorbic acid (AA) content in *Portulaca oleracea*.

Tab. 2: Phenolic compounds in the leaves of *Portulaca oleracea* in response to foliar application of different 5-ALA concentrations.

Phenolic Compounds (mg g^{-1})	5-ALA (mg L^{-1})		
	0	25	50
Catechin	$0.26^{c} \pm 0.02$	$0.37^{b*} \pm 0.04$	$0.52^{a} \pm 0.03$
Chlorogenic acid	$0.02^{a} \pm 0.005$	$0.01^{b} \pm 0.004$	$0.02^{a} \pm 0.006$
Ellagic acid	$0.02^{c} \pm 0.004$	$0.07^{b} \pm 0.005$	$0.22^{a} \pm 0.01$
Trans-ferulic acid	ND	$0.17^{a} \pm 0.02$	$0.15^{a} \pm 0.04$
Hesperedin	ND	$0.03^{b} \pm 0.004$	$0.11^{a} \pm 0.01$
Eugenol	ND	$0.24^{a} \pm 0.03$	$0.24^{a} \pm 0.02$

*Means followed by the same letter are not significantly different according to DMRT at $P \leq 0.05$. Data are mean ±standard deviation of eight replications. ND: Not detected

purslane plants (0.26 mg g^{-1}) which increased by 200% in 50 mg L^{-1} 5-ALA treated plants. Ellagic acid content in the control plant extract was 0.02 mg L^{-1} which was increased by 1100% in the 50 mg L^{-1} 5-ALA treated plants.

Moreover, the 5-ALA treatments increased the number of detected phenolics compounds in the plant to six. Trans-ferulic acid, hesperedin, and eugenol were the compounds which detected in the 5-ALA treated plants. Concentration of trans-ferulic acid and eugenol in both 5-ALA treatments were the same, however, the 50 mg L^{-1} 5-ALA treated plants had higher hesperedin in comparision with the 20 mg L^{-1} 5-ALA treated plants.

Fatty acids concentration

Two saturated fatty acids (SFA) including palmitic acid (C16:0) and stearic acid (C18:0); and two polyunsaturated fatty acids (PUFA) including linoleic acid (18:2ω6) and α-linolenic acid (18:3ω3) were found in the plant leaves. The regression models corresponding to each fatty acid in function of the studied factor are shown in Fig. 4. The fits of the regression models have high R^2 values (> 0.90) (Fig. 4). Therefore, R^2 indicate a considerable response of fatty acids to the studied factor. The regression model for the concentration of palmitic acid (Fig. 4) indicates a decrease from the applied 5-ALA. There were 11.8 and 20.4% decrease in palmitic acid for 25 and 50 mg L^{-1} of applied 5-ALA, respectively (Fig. 4).

The regression model for the concentration of stearic acid (Fig. 4) indicates a decrease from the applied 5-ALA. There were 19.46 and 32.95% decrease in stearic acid for 25 and 50 mg L^{-1} of applied 5-ALA, respectively (Fig. 4). The regression model for the concentration of linoleic acid (Fig. 4) indicates an increase from the applied 5-ALA. There were 20.28 and 37.63% increase in linoleic acid for 25 and 50 mg L^{-1} of applied 5-ALA, respectively (Fig. 4). The regression model for the concentration of α-linolenic acid (Fig. 4) indicates an increase from the applied 5-ALA. There were 3.35 and 5.67% increase in α-linolenic acid for 25 and 50 mg L^{-1} of applied 5-ALA, respectively (Fig. 4).

Mineral composition of the plant

Regardless of concentration, 5-ALA significantly increased Fe, Zn, K, Mg and N contents in purslane shoots (Tab. 3). With increasing 5-ALA concentration, an increasing trend in the mineral content of the plants was observed. In comparison to the control plants, application of 50 mg L^{-1} 5-ALA increased concentration of: Fe by 74%, Zn by 110%, and N up to 91% in the plant leaves. Purslane plants treated with 5-ALA accumulated higher Mg and K in comparison with the untreated plants. 5-ALA at the level of 50 mg L^{-1} was more effective than 25 mg L^{-1} treatment. Augmentation in shoot Mg content up to 10% and 4% and in shoot K content up to 27% and 14% were achieved by 50 and 25 mg L^{-1} concentrations of 5-ALA, respectively. The treatments caused no significant difference in shoot Ca concentration.

Antioxidative activity

The antioxidative activity in the plant extract is exhibited in Fig. 5. The linear regression equation between IC_{50} was determined as (Y, mg L^{-1}) and 5-ALA level (X, % w/v) was determined as Y = -683.01X + 3431.7 ($R^2 = 0.96$, $P \leq 0.01$) showing that IC_{50} decreased in response to foliar application of 5-ALA. The IC_{50} for the extract of 5-ALA treated plants ranged between 1465.46 ± 6.58 mg L^{-1} and 2831.49 ± 6.21 mg L^{-1}. The highest antioxidative activity (1465.46 mg L^{-1}) observed in the 50 mg L^{-1} 5-ALA treated plants, while the lowest value (2831.49 mg L^{-1}) was found in the control plants.

Organic acid contents and pH of root exudates

Without 5-ALA application, the purslane root mucilage pH was 7.30 (Tab. 4). The 5-ALA treatments brought about a significant decrease in the root exudates pH, as the lowest pH was observed at 50 mg L^{-1} 5-ALA treatment. Various organic acids were detected in the plants roots exudates. The 5-ALA treatments influenced concentration of these compounds in the root exudates. Higher exudation of organic acids was observed in the 50 mg L^{-1} 5-ALA treated plants. After foliar application of 5-ALA (50 mg L^{-1}), the highest concentration of organic acids was observed for formic acid (5.84 mg g^{-1} root DM),

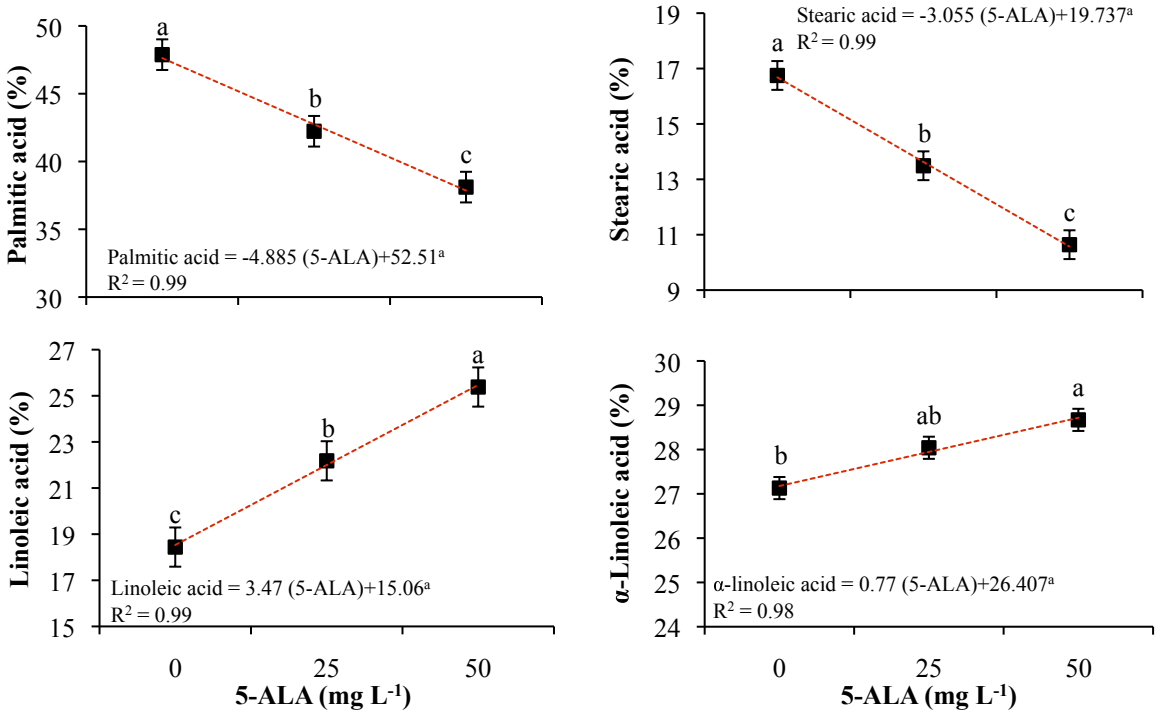

Fig. 4: Percentage of palmitic (16:0), stearic (18:0), linoleic (18:2ω6), and α-linolenic (18:3ω3) acids of the total fatty acids in leaves of *Portulaca oleracea* L. in response to foliar application of 5-ALA. Means marked by the same letter are not significantly different according to DMRT at $P \leq 0.05$. Error bars represent standard deviation.
[a] The fatty acids are expressed in percentages of the total fatty acids; (5-ALA) = applied 5-ALA (mg L^{-1}); R^2 = multiple determination coeffcient.

Tab. 3: Mineral composition of *Portulaca oleracea* shoot in response to foliar application of different 5-ALA concentrations.

Minerals (mg g^{-1} DW)	5-ALA (mg L^{-1})		
	0	25	50
N	$69.9^{c} \pm 2.06$	$101.5^{b} \pm 2.33$	$133.8^{a} \pm 2.46$
K	$488^{c} \pm 13$	$559^{b} \pm 12$	$623^{a} \pm 14$
Ca	$88.4^{a} \pm 0.96$	$88.7^{a} \pm 0.92$	$87.1^{a} \pm 0.91$
Mg	$73.6^{b} \pm 0.55$	$76.8^{ab} \pm 0.43$	$81.3^{a} \pm 0.67$
Zn	$0.77^{c} \pm 0.14$	$1.36^{b} \pm 0.13$	$1.62^{a} \pm 0.11$
Fe	$9.22^{b} \pm 0.99$	$10.88^{b} \pm 1.08$	$16.12^{a} \pm 1.12$

*Means followed by the same letter are not significantly different according to DMRT at $P \leq 0.05$. Data are mean ±standard deviation of eight replications.

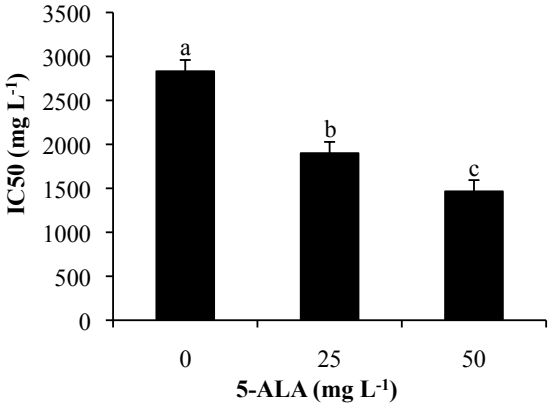

Fig. 5: The effects of different 5-ALA treatments on antioxidative activity of *Portulaca oleracea* extract measured by DPPH assay. Means marked by the same letter are not significantly different according to DMRT at $P \leq 0.05$. Error bars represent standard deviation.

followed by malic acid (5.52 mg g^{-1} root DM), oxalic acid (5.10 mg g^{-1} root DM), succinic acid (4.71 mg g^{-1} root DM), and then citric acid (3.83 mg g^{-1} root DM). Glutamic acid had the lowest (0.71 mg g^{-1} root DM) level among the root exuded organic acids (Tab. 4).

Discussion

Growth and mineral composition of the plant

Foliar application of 5-ALA is known to enhance plant growth (Akram and Ashraf, 2011; Liu et al., 2016). The present study confirmed that foliar application of 5-ALA increases shoots growth of *P. oleracea* (Tab. 1). The effect of 5-ALA on enhancing plant growth could be a result of improvement of the plant function in absorption of water and minerals. 5-ALA foliar application improved concentrations of essential minerals in the plant shoot. This was an evidence for the role of 5-aminolevulenic acid in reclaiming the cell membrane integrity and reducing root permeability (Essa, 2002). Minerals are required for osmotic adjustment, protein biosynthesis, and maintenance of root cell membrane integrity (Essa, 2002). Wei et al. (2012) suggested that exogenous 5-ALA promotes growth of young seedlings of *Brassica campestris* ssp. *chinensis* by increasing formation of amino acids and proteins due to increase in nitrogen absorption and assimilation. The definite effects of 5-ALA on improving mineral nutrients absorption and increasing plant growth may be a consequence of enhancement of plant photosynthetic capacity (Hotta et al., 1997). The effect of 5-ALA on photosynthesis rate enhancement is related to increased stomatal conductance (Naeem et al., 2011). In this regard, K plays a key role in controlling stomata and function of photosynthesis apparatus and Fe is required for biosynthesis of photosynthetic pigments (Marschner, 2011).

Tab. 4: Organic acid concentrations and pH in root mucilage of *Portulaca oleracea* in response to foliar application of different 5-ALA concentrations.

5-ALA (mg L^{-1})	pH of root exudates	Concentration of organic acids (mg g^{-1} root DM)						
		Formic acid	Succinic acid	Acetic acid	Malic acid	Oxalic acid	Glutamic acid	Citric acid
0	7.30 ± 0.02[a]	0.83 ± 0.05[c]	0.33 ± 0.03[c]	0.49 ± 0.04[c]	1.58 ± 0.02[c]	2.71 ± 0.05[c]	0.29 ± 0.05[c]	0.78 ± 0.02[c]
25	6.90 ± 0.02[b]	4.21 ± 0.09[b]	3.58 ± 0.06[b]	2.12 ± 0.06[b]	3.56 ± 0.09[b]	4.66 ± 0.01[b]	0.54 ± 0.01[b]	2.87 ± 0.05[b]
50	6.53 ± 0.04[c]	5.84 ± 0.05[a]	4.71 ± 0.09[a]	3.76 ± 0.05[a]	5.52 ± 0.05[a]	5.10 ± 0.02[a]	0.71 ± 0.01[a]	3.83 ± 0.09[a]

*Means followed by the same letter are not significantly different according to DMRT at $P \leq 0.05$. Data are mean ±standard deviation of eight replications.

The significant correlations between the concentrations of minerals in the plant shoot were not necessarily related to synergistic or antagonistic relations among them (Wei and Zhai, 2010). These correlations could be a subsequence of changes in environmental or physiological factors (Wei and Zhai, 2010). For instance, the soil used in this study was an alkaline calcareous and the elevated Fe and Zn uptake from the soil could be a result of the enhanced exudation of organic acids from the roots. The exudations by reducing rhizosphere pH and chelating the Fe and Zn ions improve their absorption from calcareous soils (Marschner, 2011). Elevated shoot Fe and Zn uptake in the 5-ALA treated plants might partly be due to increase in N uptake. Improved N nutrition status increases the activity and number of Fe-transporter proteins on the root cell membranes (Murata et al., 2008). Furthermore, 5-ALA might help Fe acquisition by purslane leaves. Aminolevulinic acid is absorbed via transport proteins containing amino acid permease 5(AAP5), amino acid permease 1 (AAP1) and lysine-histidine (LHT1) (Svennerstam et al., 2007). Our results are in agreement with Liu et al. (2016) who reported that 5-ALA application improved the contents of Cu, Zn and K in rapeseed. On the contrary, Watanabe et al. (2000) showed that 5-ALA had no effect on the uptake of Ca, Mg and K by *Gossypium* L..

In this study, the 5-ALA treatments had no significant effects on Ca concentration in the plant shoot. This observation can be explained as follows. 1) Ca absorption is a passive processand is known to be dependent on environmental conditions e.g. water availability to plant and transpiration rate (Marschner, 2011); 2) the abundance of Ca in the growing medium could supply high Ca to the plant and plants are known to pump out extra Ca from their roots. This mechanism allow them to prevent negative effects of high Ca concentrations on the normal metabolism of cells (Marschner, 2011).

Did 5-ALA affect the bioactive compounds and antioxidative activity in the plant leaves?

Application of 5-ALA significantly increased phenolics in the plant leaves; moreover, new phenolic compounds (trans-ferulic acid, hesperedin, and eugenol) were detected in the leaves of 5-ALA treated plants. In agreement, exogenous 5-ALA application has been reported to increase total phenols in date palm (Al-Qurashi and Awad, 2011) and lettuce (Aksakal et al., 2017). Extracts of many plants containing phenolics represent effective antioxidative attributes (Koleva et al. 2002). Other investigations also showed an increase in phenolic compounds of tomato (Daneshmand and Oloumi, 2014) and fuji apple (Xie et al., 2013) in response to 5-ALA treatments. 5-ALA treatment by promoting phenylalanine ammonia-lyase activity, increases the total polyphenol content of plant (Wang et al., 2016). Xu et al. (2011) demonstrated that 5-ALA treatment increased concentrations of flavonoids, anthocyanins, and polyphenolics, and promoted chalcone synthase, phenylalanine ammonia-lyase, and chalcone isomerase activities in *Ginkgo biloba*. In addition to catechin, the predominant phenolic compound in purslane extract, 5-ALA treatments brought a large increase in ellagic acid content in the plant extract (Tab. 2). Both compounds are effective natural ROS scavengers in plants. Catechin may also act indirectly as antioxidant through its effect on enzyme activities and transcription factors (Higdon and Frei, 2003). Ellagic acid also is an important antiproliferative agent, which prevents the DNA binding of carcinogens such as nitrosamines (Mandal and Stoner, 1990) and polycyclic aromatic hydrocarbons (Teel et al., 1986). More importantly, both of these phytochemicals have exhibited antiangiogenesis activity *in vitro* and *in vivo* (Wang et al., 2012). Antiangiogenesis is of important part of the treatment strategies in a variety of neoplasms such as breast, colon, lung, and kidney cancers.

In addition to polyphenols, 5-ALA significantly increased ascorbic acid (AA) that is synthesized from hexoses through the shikimate and phenylpropanoid pathways (Xu et al., 2011). AA has antioxidative activity and has the ability to inhibit cancer and cardiovascular disease (Uddin et al., 2014). In parallel with enhancement of shoot polyphenolics and AA content, foliar application of 5-ALA increased antioxidative activity in shoots of purslane. Our findings are in agreement with Akram and Ashraf (2011) who illustrated that 5-ALA can efficiently enhance the antioxidative activity in *Helianthus annus* L.. Akram and Ashraf (2013) also demonstrated that application of low levels of 5-ALA could considerably increase the levels of non-enzymatic antioxidants like AA and glutathione in leaves of ginkgo.

Accumulation of phenolics and AA could be the reason of enhancement in the antioxidative activity in purslane leaves (Aksakal et al., 2017). The noticeable raises in antioxidant activity of kudzu (*Pueraria phaseoloides*) following 5-ALA treatment greatly enhanced the medicinal value of the plant (Xu et al., 2010). Previous studies also reported that application of low concentration of 5-ALA (1 mg L^{-1}) improved antioxidants in *Brassica napus* (Naeem et al., 2011) and *Spinacia oleracea* (Nishihara et al., 2003). Therefore, application of 5-ALA to increase content of bioactive compounds could be a useful method to enhance antioxidant capacity and pharmaceutical value of purslane.

Palmitic acid (37.1-47.9%) was the fatty acid with the highest concentration in purslane leaves. α-linolenic acid (18:3ω3), between 27.1 and 28.7 %, and linoleic acid (18:2ω6), between 18.4 and 25.4% were the other major fatty acids in the plant's leaves. Stearic acid (C18:0), between 10.64 and 16.75%, had the lowest concentration among the fatty acids. These results coincide with those reported by Montoya-Garcia et al. (2018). Meanwhile, Mera-Ovando et al. (2014) reported three fatty acids in purslane in the following order of concentrations: linoleic > αlinolenic > arachidonic. However, other researchers reported a higher concentration of α-linolenic acid, followed by palmitic and linoleic acids in the plant (Siriamornpun and Suttajit, 2010). The omega-6/omega-3 ratio in purslane leaves was 0.68 without 5-ALA application. This value increased to 0.79 and 0.88 with the application of 25 and 50 mg L^{-1} 5-ALA. But, a lower ratio of omega-6/omega-3 fatty acids is more desirable to decrease the risk of chronic diseases, such as cardiovascular, cancer, or inflammatory diseases. Sanchez and Harwood (2002) stated that the modifications in the fatty acids profile is due to increase or inhibition of oleate desaturase activity during the biosynthesis of triacylglycerol.

Conclusion

Our results showed that foliar application of 5-ALA increased shoot Fe, Zn, N, Mg and K contents and improved growth of *Portulaca oleracea*. 5-ALA also increased fatty acids, polyphenols content and antioxidant capacity of the plant. Therefore, 5-aminolevulinic acid appears to provide an opportunity to increase yield and quality of leafy vegetables. Furthermore, it can be used at low concentrations to improve pharmaceutical and nutritional values of medicinal plants. How this information might be used commercially still needs to be resolved. Moreover, to realize the mechanism of action of 5-ALA specified studies have to be carried out.

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