ACTA SCIENTIARUM POLONORUM

Acta Sci. Pol. Hortorum Cultus, 17(5) 2018, 179–190

www.acta.media.pl 1644-0692 e-ISSN 2545-1405 DOI: 10.24326/asphc.2018.5.16

ORIGINAL PAPER

Accepted: 3.04.2018

EFFECT OF IRON NANO CHELATE ON ANTIOXIDANT ACTIVITY, POLYPHENOLIC CONTENTS AND ESSENTIAL OIL COMPOSITION OF *Portulaca oleracea* L.

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ABSTRACT

In the present study, using ultrasonic irradiations, a novel nano-sized iron complex has been prepared from aminolevulinic acid and iron(III) nitrate under greenhouse conditions. The obtained Fe nano-sized complex has been characterized by two methods of Fourier-transform infrared spectroscopy (FTIR) and EDX spectra (Energy-dispersive X-ray spectroscopy). Also, the morphology and size of the nano-complex were determined using transmission electron microscopy (TEM) and it showed an acceptable size in the nano range (5–20 nm). In this work, purslane plants were supplied with Fe(III)–aminolevulinic acid (Fe-ALA) as a new nano-sized complex and Fe-EDDHA [Fe-ethylenediamine-N,N'-bis(2-hydroxyphenylacetic acid)]. The mineral nutrients concentrations, total phenolic, ascorbic acid contents and antioxidant activity were the highest in plants treated with Fe-ALA nano-complex. Catechin was the predominant phenolic compound in all treated plants. Fe nano-complex at the rate of 0.2% induced extra high phenolic compounds value. Shoot Fe, Zn, N, Mg, Ca and K contents were also higher in Fe nano-complex treated plants than the control and in plants that were treated by Fe-EDDHA. Overall, the nutritional and pharmaceutical quality of *Portulaca oleracea* improved the use of the nano-sized Fe-ALA complex as a new iron source.

Key words: antioxidant activity, iron, mineral nutrients, nano chelate, purslane, polyphenolic content.

INTRODUCTION

Nowadays, one of the areas of science that plays an important role in chemistry and agriculture is nanotechnology. In other words, nanotechnology exposed modern occasions to reduce the environmental protection cost and improve the nutrient use efficiency [Naderi and Danesh Shahraki 2011]. Nanostructures are one of the most important branches of nanotechnology. Among all existing nanostructures, nanoparticles, that have a very high surface area to volume ratio, are of great scientific interest. Synthesis of nano-sized metal complexes is receiving a great attention in recent years owing to their vast applications and unique properties in various sciences. Also the green synthesis of these nano-complexes has attracted more attention for environmental remediation [Wei et al. 2016].

Antioxidants are naturally found in vegetables along with different phytochemicals. Antioxidants, that have gained popularity for the dominance of phenolic compounds in them, are very suitable for decreasing the oxidative stress. Therefore, they are recognized as the dominating factor in plants' anti-

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oxidant activity. Antioxidants are able to perform free radicals scavenging due to hydrogen contribution when their assault to biological parts and cells has not started yet. Therefore, they are deemed necessary for the wealth and verification of an ideal wellbeing [Percival 1998]. Plants contain a broad spectrum of different valuable compounds such as flavonoids (anthocyanins, flavones, flavonols, etc.) and some non-flavonoids categories (phenolic acids, lignins, stilbenes, terpenoids, etc.) as phenolic components. The aforementioned compounds differ in terms of structure; for instance, phenolic hydroxyl groups' position and their corresponding number, triggering disparity in their anti-oxidative role [Knekt et al. 1996].

The common purslane (*Portulaca oleracea* L.) in Portulacaceae family includes many species the number of which exceeds 120, that are succulent herbs and bushes. Various countries use the aerial plant parts as antiseptic, febrifuge, diuretic, vermifuge and antispasmodic agents [Xiang et al. 2005]. In the Middle East countries, *Portulaca oleraceae* is eagerly consumed as a vegetable. The interest in the plant as food is usually drawn to their leaves and stems as they are edible and they have an acidic and salty taste as *Spinacia oleracea* L. It is common, self-compatible, rapidly grows and produces lot of seeds [Liu et al. 2000]. Plentiful purslane collections are cultivated in a widespread diversity of districts and climates. It is known to have advanced nutrition value rather than other vegetables, due to a boundless source of ascorbic acid, phenolics and antioxidants [Liu et al. 2000].

The management of nutritive plants forms one of the causes that influences the chemical arrangements and quality of pharmaceutical plants and vegetables, with microelements that play a significant role in their production [Yousefzadeh and Sabaghnia 2016]. Their availability, price rate and efficiency of domestic fertilizer sources that employ modern technology has a two-fold benefit. They are economical to produce resulting in decreased fertilizer loss and important in reducing the environmental pollution. Iron has played many roles in the activity of many antioxidant enzymes, chlorophyll and thylakoid synthesis, chloroplast development, RNA synthesis and enhancing the photosystems performance [Ghasemi et al. 2014]. Synthetic Fe-chelates, including Fe–EDTA and Fe–DTPA, are currently utilized to raise the availability of Fe in soil and to preserve its necessary amount [Vadas et al. 2007]. Synthetic Fe-chelates are operative bases of Fe, particularly in alkaline and calcareous soil [Rodriguez-Lucena et al. 2010]. The superlative prevalent iron source applied in agriculture is Fe (III)-EDTA chelate [Vadas et al. 2007]. Even though EDTA preserves the solubility of Fe, after metal uptake by plant, free ligand concentration increases and therefore it leads to higher complex formation possibility of the free ligands with various microelements (such as Cu, Mn, and Zn) decreasing the availability and plant uptake of the metals [Vadas et al. 2007]. Furthermore, EDTA and DTPA chelates are sensitive to photodegradation. The half-life of Fe–DTPA and Fe–EDTA chelates exposed to sever sunlight situations are eleven and eight minutes, correspondingly [Metsarinne et al. 2004]. Photodegradation of synthetic ligands leads to the creation of a kind of compounds that are destructive for growth of plants and lessen availability of Fe for plants due to its precipitation [Vadas et al. 2007, Metsarinne et al. 2004].

In current study, we introduce nano Fe-amino chelate, synthesized through the process of Fe complexation with certain 5-aminolevulinic acid as a novel Fe source for soil treatment. Amino acids organize Fe ion via their own amine and carboxylate groups. Fe-amino acid chelates are moreover steady in the soil [Ghasemi et al. 2014].

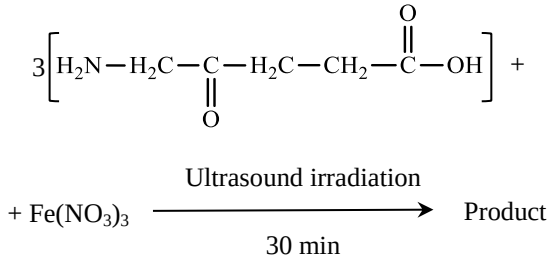
There is a shortage of data in regards to how the blend of amino acids and Fe and their nanoscale quality affect the therapeutic and aromatic plants. Nanoparticles that have broad surface territory and negligible size are needed to be the suitable chance as a fertilizer in plants. Both sulfate salts and chelated Fe (with ethylene diamine tetra acetic acid, EDTA) are used in soil and foliar applications by farmers, in spite of their low efficacy. Similarly, hypothesis was that nano chelate as the novel Fe compost source is highly likely effective in developing polyphenol substance and the antioxidant activity of plants applied in medicine. This hypothesis was tested through the inspection of impacts of the foliar use of synthetic

Fe-amino nano chelate i.e. [$Fe(ALA)_3$] on growth, phenolic compounds, antioxidant activity and nutritional status of purslane plant in an efforts to improve its application for the public consumption.

MATERIAL AND METHODS

Analytical grade chemicals were utilized. For nanoparticles characterization, the transmission electron micrographs (TEM) was acquired utilizing TEM device (100 kV Philips, EM208). The EDX and FTIR spectra were obtained utilizing SEM-EDX analyzer (Tescan Vega II, with a Rontec detector) and Tensor II FTIR spectrometer, respectively.

For the synthesis of the nano-sized Fe-ALA complex, first of all, at room temperature, 5-aminolevulinic acid (2 mmol) was dissolved in deionized water (5 mL). A solution of $Fe(NO_3)_3$ (1 mmol) in 3 mL distilled water was added to this solution. The chemical reaction was exposed to sonication using an ultrasonic probe. The whole mixture was subject to stable oscillation of 90% for 30 min in air atmosphere. The mixture was chilled off to ambient temperature and the solvent was evaporated in the air flow overnight. After that under vacuum for 18 h, the achieved dark brown powder was dried.



A greenhouse trial was completed at the Shiraz Payame Noor University situated in Golestan town (29°36′N and 52°32′E, 1490 m above sea level). Soil in this study was a fine topsoil loam taken from 0 to 30 cm of a virgin soil. Some physicochemical properties of the soil are displayed as follows: pH paste: 7.1, EC_e: 1.2 dS m^{-1}, CEC: 10 Cmc kg^{-1}, organic C: 8.9 g kg^{-1} soil, N: 0.07%, P: 13 mg kg^{-1} soil, K: 59 mg kg^{-1} soil, DTPA-extractable Fe: 2.21 mg kg^{-1} soil. Regarding the basic acute rate for the DTPA-extractable soil Fe (2 mg kg^{-1}) [Fageria et al. 1990], the soil was not extremely deficient in available Fe. Nitrogen and phosphorus 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 as NH_4NO_3, KH_2PO_4, $CuSO_4$ $5H_2O$, $ZnSO_4$ $7H_2O$ and $MnSO_4$ H_2O, respectively. Soil was placed in 8-liter plastic pots at the rate of 7.5 kg pot^{-1}. Twenty seeds of purslane (*Portulaca oleracea* L.) were planted in the pots and irrigated with deionized water twice a week to the field capacity. After fifteen days, the plants were thinned to 10 uniform stands in each pot. At two phases: after thinning and upon the onset of inflorescence appearance, Fe was sprayed at the rates of 0.1 and 0.2% (w/v) in the forms of synthetic Fe-ALA nano-complex (n[$Fe(ALA)_3$]) and Fe-EDDHA. Deionized water was also sprayed as control. Plants were ripped 12 weeks after planting. In shade, plant samples were desiccated and subjected to analyses.

Methanol extracts of *Portulaca oleracae* L. samples were prepared by the method described by 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 extracts were made in a rotary evaporator for ten minutes. By weighing, the yields of fine powders were recorded. The powders were maintained at −18°C before use. Needed level of powder in methanol was arranged before each measurement, and then total phenol content and the antioxidant activity were evaluated.

Polyphenol extraction was carried out in a view of past reports with few changes [Justesen et al. 1998]. HPLC examination was done on an Agilent Technologies 1200 series (Germany), equipped with a Zorbax eclipse (XDB) C_{18} (5µm (ID), 4.6 × 150 mm (FT)) and a photodiode cluster identifier. At 230 and 280 nm, elution was observed. By changing the methanol to formic acid proportion, elution was performed.

Using the Folin-Ciocalteu reagent, the total phenolic compounds (TPC) in the purslane extract were measured. 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 Folin-Ciocalteu's reagent (diluted

10 times) were added to 300 mL of samples. Mixtures of above compounds were shaken and left at ambient temperature for thirty minutes. Then, the mixture absorbance was measured at 765 nm by a spectrophotometer instrument (Varian 220, Australia). The measurement was performed 3 times. We tried to present the total phenol content as gallic acid (GAE) equivalent in 100 g fresh sample. For all TPC values, by subtracting the ascorbic acid content (AAC) from the total phenol value, corrections were made.

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

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

where A_{sample} and A_{blank} are absorption values of the test solution (t = 30 min) and the blank reaction (t = 0 min). DPPH (without plant extract) and methanol were used as control and blank, respectively.

Samples were desiccated for 72 h at 70°C in an oven. Dried purslane samples were powdered and kept in plastic vials. Nitrogen concentration of shoot was determined by Autotech (Model 300). Then samples (ca. one gram per replicate) were combusted for 6 h at 550°C. The white ash powder was added into 5 mL of 2 M hot hydrochloric acid, filtered into a 50 mL volumetric flask and, made up with water to 50 mL. Iron and zinc analyses were done applying an atomic absorption spectrophotometer (Model Varian 220, Australia). By flame photometer, the concentrations of sodium and potassium in plant extracts were analyzed. Magnesium and calcium concentrations were determined in plant extracts using inductively coupled plasma-mass spectrometry (ICP-MS, X-Series II; Thermo Fisher Scientific Inc., Waltham, MA, USA). At last, via multiplying the weight of dry matter by nutrient minerals concentrations, the total amount of nutrients was calculated.

The statistical analysis of data was carried out using the SPSS software (version 20) for checking the significance of distinctive treatments, through Duncan's multiple range test at the level of 5% probability to compare differences between the mean values.

RESULTS

Our prime trials were concentrated on the specifications of the nano-sized iron complex. The FT-IR spectroscopy was used to determine the vibration characteristics of chemical functional groups. The FTIR of the resulted material shows an absorption pattern similar to amino acid ligands in 500–4000 cm^{-1} region. In comparison with the free 5-aminolevulinic acid, the N-H vibrational bands in Fe-ALA nano-complex were shifted toward higher frequency. The absorption band around 1575–1615 cm^{-1} in the 5-aminolevulinic acid spectrum was attributed to the C = O group and emerges to be transferred to higher wave numbers in the Fe-ALA nano-complex spectra, which includes the COOH group in the covalent bonding to the iron(III) ion. In accordance with the energy dispersive X-ray analysis (EDX), the attendance of iron is illustrated in the Fe-ALA nano-complex. The EDX spectrum also displays carbon, oxygen and nitrogen signals which exist in the Fe-ALA nano-complex (Fig. 1). The transmission electron microscopy (TEM) image indicates that the iron particle size is in the span of 5–20 nm. The TEM image of the Fe-ALA nano-complex showed that the nanoparticles of Fe with close globular morphology were organized onto the pectin surface with comparatively fine monodispersity (Fig. 2).

Fresh weight (FW), dry weight (DW), relative water content (RWC) and shoot length was measured

over twelve weeks (Tab. 1). The shoot dry weight, fresh weight and length of purslane plants supplied with Fe-ALA nano-chelates was greater than those supplied with Fe-EDDHA (Tab. 1). The shoot growth parameters of purslane plants supplied with n[Fe(ALA)$_3$] at 0.2% level were significantly greater than those supplied with other rates of Fe fertilizers. Both types of iron fertilizers significantly reduced relative water content, as untreated (control) plants had the highest level of water content (Tab. 1).

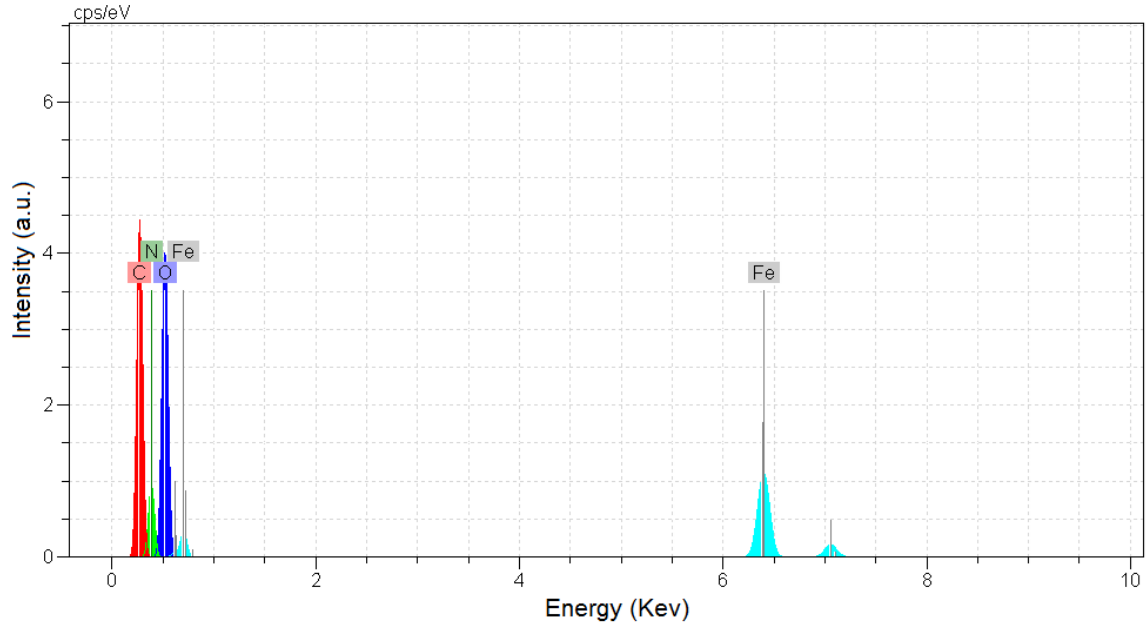


Fig. 1. The EDX spectrum of Fe-ALA nano-sized complex

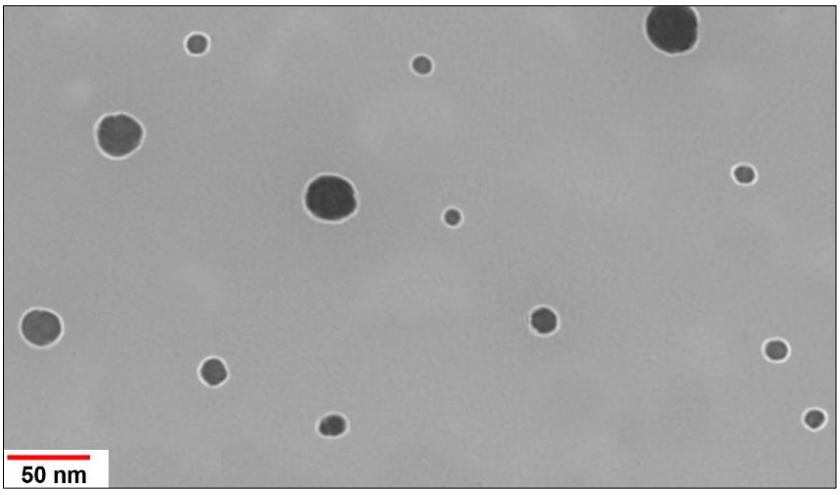


Fig. 2. The TEM image of Fe-ALA nano-sized complex

Table 1. Fresh weight (FW), dry weight (DW), shoot length and relative water content (RWC) of purslane at different iron treatments

Treatments	FW (g)	DW (g)	Shoot length (cm)	RWC (%)
n[Fe(ALA)$_3$] 0.1%	48.37^{b*} ±1.03	5.44^{b} ±0.043	27.25^{b} ±0.85	86^{c} ±1.82
n[Fe(ALA)$_3$] 0.2%	55.71^{a} ±1.22	7.68^{a} ±0.037	33.59^{a} ±0.74	85^{c} ±1.26
Fe-EDDHA 0.1%	38.13^{c} ±0.92	3.29^{c} ±0.047	26.81^{b} ±0.68	90^{b} ±1.62
Fe-EDDHA 0.2%	47.59^{b} ±1.26	3.66^{c} ±0.028	31.76^{a} ±0.84	92^{b} ±1.37
Control	31.21^{d} ±0.85	1.23^{d} ±0.035	19.39^{c} ±0.77	96^{a} ±1.74

* Means followed by the same letter within a column are not significantly different P ≤ 0.05 (Duncan)

Table 2. Total phenolics and ascorbic acid contents for *Portulaca oleracea* at the different iron treatments

Treatments	TPC (mg GAE 100 g^{-1})	AAC (mg 100 g^{-1})
n[Fe(ALA)$_3$] 0.1%	367.26^{a*} ±6.57	80.52^{a} ±4.24
n[Fe(ALA)$_3$] 0.2%	353.13^{b} ±7.41	78.92^{ab} ±3.15
Fe-EDDHA 0.1%	321.06^{c} ±8.33	76.34^{bc} ±3.73
Fe-EDDHA 0.2%	282.19^{d} ±6.89	74.14^{c} ±2.87
Control	223.33^{e} ±5.71	66.22^{d} ±3.18

Means followed by the same letter within a column are not significantly different P ≤ 0.05 (Duncan)

Table 3. Phenolic compounds of *Portulaca oleracea* affected by iron fertilizers

Phenolic compounds (mg g^{-1})	Foliar-applied Fe treatments				Control
	n[Fe(ALA)$_3$] 0.1%	n[Fe(ALA)$_3$] 0.2%	Fe-EDDHA 0.1%	Fe-EDDHA 0.2%	
Catechin	0.57^{a*} ±0.06	0.44^{b} ±0.04	0.33^{c} ±0.03	0.29^{c} ±0.04	0.26^{c} ±0.02
Chlorogenic acid	0.05^{a} ±0.003	0.03^{b} ±0.003	ND	ND	0.02^{b} ±0.002
Trans-ferulic acid	ND	0.15^{a} ±0.04	0.15^{a} ±0.03	0.13^{a} ±0.02	ND
Hesperedin	0.13^{a} ±0.03	0.11^{b} ±0.01	0.05^{c} ±0.002	ND	ND
Ellagic acid	ND	0.30^{a} ±0.005	ND	0.26^{a} ±0.02	ND

* Means followed by the same letter within a row are not significantly different P ≤ 0.05 (Duncan)
ND – not detected
Data are mean ±standard deviation of eight replications

Table 4. Mineral concentrations in *Portulaca oleracea* at different iron fertilization

Minerals (mg g^{-1} DW)	Foliar-applied Fe treatments				Control
	n[Fe(ALA)$_3$] 0.1%	n[Fe(ALA)$_3$] 0.2%	Fe-EDDHA 0.1%	Fe-EDDHA 0.2%	
N	105.61^{b*} ±2.61	143.28^{a} ±2.76	83.73^{d} ±1.99	91.28^{c} ±2.55	68.92^{e} ±2.06
Mg	78.4^{b} ±0.55	97.51^{a} ±0.72	69.13^{c} ±0.78	81.67^{b} ±0.59	68.22^{c} ±0.61
Ca	65.44^{c} ±1.02	71.29^{a} ±0.81	67.81^{bc} ±1.12	69.59^{ab} ±0.94	61.79^{d} ±0.76
Fe	11.72^{b} ±0.78	14.27^{a} ±0.82	8.92^{c} ±0.67	11.84^{b} ±0.78	5.34^{d} ±0.79
Zn	1.09^{b} ±0.11	1.56^{a} ±0.08	0.82^{c} ±0.05	0.97^{bc} ±0.07	0.66^{d} ±0.04
K	562^{b} ±11	617^{a} ±13	525^{c} ±16	551^{b} ±10	423^{d} ±12
Na	82.1^{a} ±0.42	81.4^{a} ±0.28	79.13^{a} ±0.61	80.22^{a} ±0.56	82.33^{a} ±0.51

* Means followed by the same letter within a row are not significantly different $P \leq 0.05$ (Duncan)
Data are mean ±standard deviation of eight replications

Table 5. Antioxidant activity of *Portulaca oleracea* affected by different Fe treatments

Antioxidant activity	Foliar-applied Fe treatments				Control
	n[Fe(ALA)$_3$] 0.1%	n[Fe(ALA)$_3$] 0.2%	Fe-EDDHA 0.1%	Fe-EDDHA 0.2%	
IC_{50}^{*} for ROS scavenging (mg mL^{-1})	1.38 ±0.08	1.57 ±0.2	2.10 ±0.09	2.46 ±0.4	2.82 ±0.5

* Concentration of extract required to scavenge ROS (reactive oxygen species) by 50%
ROS – reactive oxygen species

The total polyphenolics and ascorbic acid content in aerial parts of purslane was higher at n[Fe(ALA)$_3$] treatments compared with the Fe-EDDHA treatments (Tab. 2). Purslane plants supplied with Fe-ALA nano-complex at the level of 0.1% (w/v) had the highest TPC and AAC levels compared with those supplied with higher concentration or Fe-EDDHA. Total phenolics was 367.26 mg GAE 100 g^{-1} and ascorbic acid content was 80.52 mg 100 g^{-1} in plants supplied with 0.1% (w/v) [Fe(ALA)$_3$] nano-particles.

In this study, there were only 2 phenolic compounds detected in the control plants. Fe-ALA nano-complex increased the number of detected compounds to five, including catechin, chlorogenic acid, trans-ferulic acid, hesperedin and ellagic acid (Tab. 3). Fertilization with Fe-EDDHA raised the number of phenolics to 3 compounds compared with non-treated plants, which produced 2 compounds. Catechin was the predominant phenolic compound in all purslane plants, contributing from 0.57 mg g^{-1} in treated plants with 0.1% n[Fe(ALA)$_3$] to 0.26 mg g^{-1} in the control plants.

Iron, Zn, K, Ca, Mg and N contents in purslane shoots were improved significantly in treatment where Fe fertilizer (regardless of Fe source) was applied in comparison with the control (Tab. 4). No significant differences were observed between shoot Na concentrations in various Fe treatments. Among all treatments, Fe-nano complex at the level of 0.2% (w/v) remained the best as compared to other treatments, which increased Fe up to 2.6 fold, over the control (Tab. 4). Plants supplied

with n[Fe(ALA)$_3$] at 0.1% and Fe-EDDHA at 0.2% also showed raising shoot Fe content up to 119% comparing with control. Zinc concentration in purslane shoots ranged from 0.66 mmol kg^{-1} DW in control to 1.56 mmol kg^{-1} DW in n[Fe(ALA)$_3$] treated plants at 0.2% (w/v). Zn concentration increased up to 136% and 47% by applying 0.2% of n[Fe(ALA)$_3$] and Fe-EDDHA, respectively, over control. Iron applied at 0.2% (w/v) of n[Fe(ALA)$_3$] and Fe-EDDHA enhanced N concentration up to 107% and 32%, respectively, as compared with intact plant (control). The highest shoot N content was achieved by application of Fe nano-complex at 0.2% concentration. Purslane plants supplied with Fe(III)–ALA nano-chelate accumulated higher shoot Mg, Ca and K in comparison with those supplied with Fe–EDDHA. Fe nano-complex at the level of 0.2% was more effective than the lower one. Augmentation in shoot Mg content up to 43% and 19%, in shoot Ca content up to 15% and 12% and in shoot K content up to 46% and 30% were achieved by 0.2% concentration of n[Fe(ALA)$_3$] and Fe-EDDHA, respectively.

Plant antioxidant activity is the result of the active compounds present in them. Antioxidant activity of purslane extract is exhibited in Tab. 5. The IC_{50} values for the extract of purslane plant that was treated with Fe fertilizers in the current trial was assessed and ranged between 1.38 ±0.08 mg mL^{-1} and 2.82 ±0.5 mg mL^{-1} showing the greatest antioxidant activity (1.38 mg mL^{-1}) displayed by the purslane treated with 0.1% n[Fe(ALA)$_3$], whereas the lowest (2.82 mg mL^{-1}) was found in the control. These findings illustrated that purslane contains potent antioxidant activity and can be used as natural antioxidants for pharmaceutical purposes.

DISCUSSION

Results obtained from several studies indicate the definite effect of amino acids application on enhancing certain crops growth [Hoque et al. 2007, Kursat et al. 2007]. A possible reason for the effect of Fe–amino acid nano-chelate on improving growth of purslane plants is maintaining hormones balance within plant tissues [Nassar et al. 2003]. Iron nano-fertilizer has stimulatory effects on plant growth. The stimulating effect of Fe nano-chelate fertilizer application on peanut shoot growth [Rui et al. 2016], mung bean [Karimi et al. 2014], saffron [Maleki Farahani et al. 2015] and poinsettia [Kaviani et al. 2016] plants has been reported.

In the present study, Fe nano-complex foliar application significantly improved mineral nutrient contents in shoots of purslane. Mineral ions accumulation in plant cells improves osmotic regulation, enhances synthesis of protein, and maintains membrane integrity of root [Essa 2002]. Cuin and Shabala [2007] investigated that predominant amino acids diminished K leakage of root by ameliorating integrity of cell membrane and root K influx regulation. Enhanced shoot potassium and nitrogen contents of purslane plants supplied with Fe(III)–ALA chelate in comparison with those treated with Fe–EDDHA confirm the amino acid (5-aminolevulinic acid) role in the improvement of cell membrane integrity and functioning. Elevated shoot Fe and Zn uptake in purslane plants supplied with Fe-ALA nano-chelate might partly be due to the increase in N uptake. Improved N nutrition status increases the activity and number of Fe-transporter proteins on the cell membranes of root and as a result, enhances the root Fe uptake [Murata et al. 2008]. 5-ALA might help in 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]. The Fe–amino acid nano-chelate uptake mechanism is still unclear whether Fe–amino acid nano-chelate can enter into the root cell cytoplasm due to its size as un-dissociated complex or it dissociates as free amino acid and Fe at the surface of root and passes through the cell membrane individually. This suggestions need to be tested using isotopic (labeled Fe and N) studies. According to the diameter of Fe–ALA nano-chelate molecules estimated using TEM (Fig. 2), they are smaller than the cell wall pore diameter (less than 5 nm). Thus, no limitation seems to be on the movement of Fe–ALA nano-chelate by these free spaces to the cell membrane.

A class of antioxidant agents which act as free radical scavengers is phenolic compounds. They are responsible for antioxidant activity in medicinal

plants. Extracts of many plants containing phenolics as effective antioxidants attribute and inhibit damage of free radical [Koleva et al. 2002]. Influence of antioxidants of phenolic compounds on human health has been demonstrated due to their physiological activity, including antimutagenic, antitumor and antioxidant properties [Li et al. 2006]. Potential oxidative stress inhibition of phenolic compounds found in dietary and medicinal plants [Manach et al. 2004] and phenolic compounds from many traditional Iranian medicines showed both antioxidation and antiglycation properties. To scavenge reactive oxygen species (ROS) and OH, phenolic compounds can be used directly [Omidbeigi 2008].

During the growth of a plant, the micronutrient availability has significant ability to result phenolic accumulation [Parr and Bolwell 2000]. Benzon et al. [2015] illustrated that in white rice cultivars, the TPC can be enhanced through application of nano-fertilizer. In another study, Alizadeh et al. [2010] implied that the highest total phenolic content was found in 1000 mg/plant fertilizer treatment. The results of current study determined that total phenolic content was associated with the antioxidant activity and ascorbic acid content. The correlation coefficients obtained were 0.957 (antioxidant activity) and 0.974 (ascorbic acid content). Erkan et al. [2011] revealed a nearby correlation between total phenolic content and radical scavenging activity of extract from various natural sources. Ascorbic acid (vitamin C) has been reported to possess antioxidant activity, because of its potential to neutralize free radicals, and having the ability to inhibit cancer and cardiovascular disease [Uddin et al. 2014].

Catechin is a flavan-3-ol, and belongs to the group of flavonoids. Catechin is effective ROS scavenger and may also act indirectly as antioxidant through its effect on enzyme activities and transcription factors [Higdon and Frei 2003]. Nano-iron application had outstandingly positive effects on phenolics content. Nano-Fe complex at the rate of 0.1% incited significantly higher phenolics in comparison with control and plants treated with Fe-EDDHA fertilizer (Tab. 3). Similarly to our findings, Njogu et al. [2014] displayed that foliar application of microelements on tea plants increased polyphenol contents. Enhancing the polyphenols by the application of iron on leaves is due to the increasing leaf activity since this nutrient is applied just on the sites where photosynthesis takes place, hence this element can be rapidly transformed into compounds necessary for plant. Latest studies showed that foliar application of fertilizer increased the total leaf polyphenols, which was ascribed to the attendance of microelements in the foliar fertilization [Obatolu 1999]. Rui et al. [2016] reported that Fe nano-particles induced protection mechanisms to scavenge reactive oxygen by increasing the polyphenols in peanut leaves. Adversely, a significant decrease in polyphenol contents in maize grain was recorded when Fe was applied in soil [Ramzani et al. 2016].

Along with enhancement of shoot polyphenolics content, exposure to Fe nano-complex increased antioxidant activity in shoots of purslane. In agreement with our conclusions, Benzon et al. [2015] demonstrated that the nano-fertilizer application enhanced DPPH scavenging activity and antioxidant potential in rice. Delfani et al. [2014] revealed that application of nano-fertilizers was supplemental; their superior absorption by plant cells in some way supplied sufficient nutrients to increase antioxidant activities in black-eyed pea. The recent past studies showed that nano-scale fertilizer in peanut [Prasad et al. 2012] and soya bean [Lu et al. 2002] enhanced the antioxidant activity. In various plants, phenolic compounds and ascorbic acid have been shown to be correlated with antioxidant activity [Lopez-Velez et al. 2003]. The antioxidant activity of polyphenols as prominent antioxidants have been ascribed to different mechanisms, across which there is the transition of ion binding catalysts, prevention of chain initiation, blockage of successive hydrogen abduction, decomposition of peroxides, radical scavenging and lessening the capacity [Yildirim et al. 2001]. Increasing polyphenolics and ascorbic acid contents by Fe-ALA nano-complex application could be a useful method to enhance antioxidant activity and pharmaceutical properties of purslane plants.

CONCLUSION

An effective means with high site specificity and reduced collateral damage characterizes the nano-

particle fertilizer. Size of nanoparticle plays a significant role in reactivity and in behavior. Our results showed that foliar application of Fe(III)-aminolevulinic acid nano-chelate (as Fe source) increased shoot Fe, Zn, N, Mg, Ca and K contents and improved growth of purslane plants in comparison with Fe–EDDHA. It seems that maintaining the root cell membrane integrity was the main reason of high nutrients uptake under n[Fe(ALA)$_3$] treatments. Improved N nutrition status as well as elevated shoot growth could partly explain the positive effect of Fe–ALA nano-chelate in improving antioxidant activity and polyphenols content of purslane. In accordance with the results acquired, it is assumed that Fe(III)–aminolevulinic acid nano-chelate can be used as a novel chelate to supply Fe and to increase pharmaceutical and nutritional values of very popular Iranian vegetable known as *Portulaca oleracea* (in Persian called Khorfeh). To realize the mechanism of action of nano-scale materials, specific studies have to be carried out. In addition, the most effective application method is required to be appraised.

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