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## ANALYTICAL CHEMISTRY | RESEARCH ARTICLE

# Determination of the antioxidant activity of *Limoniastrum feei* aqueous extract by chemical and electrochemical methods

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**Abstract:** The total flavonoids (TF), total phenolics, and antioxidant activity of *Limoniastrum feei* aqueous extract were investigated. The results show that *Limoniastrum feei* contains  $200.28 \pm 2.75$   $\mu\text{g}$  of total phenolic in 1 mg of dry extract, expressed as gallic acid equivalents. The TF represent  $54.77 \pm 3.21$   $\mu\text{g}/\text{mg}$ , expressed as Quercetin equivalents. The antioxidant activity of extracts has been evaluated by chemical and electrochemical methods. In the reducing power and total antioxidant capacity tests, the antioxidant activity of extracts was expressed as Ascorbic acid equivalents where the aqueous extract has an equivalent capacity of  $233.39 \pm 4.23$  and  $112.4 \pm 1.97$   $\mu\text{g}$  for 1 mg, respectively. In DPPH• radical trapping test, the IC 50 was equal to  $0.58 \pm 0.03$  mg/ml. The cyclic voltammetry of the extract indicates one oxidation irreversible peak at approximately 300–320 mV/(Ag/AgCl). The superoxide scavenging assay of *Limoniastrum feei* aqueous extract showed an average activity of order  $61.46 \pm 2.51\%$  at 0.5 mg/ml doses.

### ABOUT THE AUTHORS

Professor Hassan Y. Aboul-Enein is a Professor at the Pharmaceutical and Medicinal Chemistry Department, National Research Center in Cairo, Egypt. He is the author and co-author of over 1000 refereed articles and 93 reviews, 55 book chapters, 8 books. He is an Editorial Board member of some 22 peer reviewed journals.

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Professor Aboul-Enein has been a visiting professor to several universities and research institutions in several countries.

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### PUBLIC INTEREST STATEMENT

Natural antioxidants are important keys to protect life and health from the damage caused by reactive oxygen and nitrogen species-induced oxidative stress. Some plants contain a high amount of polyphenols which are very powerful natural antioxidants. An experimental protocol was designed to investigate the total flavonoids (TF), total phenolics, and antioxidant activity of *Limoniastrum feei* aqueous extract using different methods. The results show that *Limoniastrum feei* contains  $200.28 \pm 2.75$   $\mu\text{g}$  of total phenolic in 1 mg of dry extract, expressed as gallic acid equivalents. The TF represent  $54.77 \pm 3.21$   $\mu\text{g}/\text{mg}$ , expressed as Quercetin equivalents. The antioxidant activity of extracts was good to be considered to be use as a natural supplement after adequate pharmaceutical formulations and pharmacological evaluation *in vivo*.

**Subjects: Chemistry; Natural Products; Pharmaceutical Science**

**Keywords: natural antioxidants; *Limoniastrum feei*; total phenolics; total flavonoids; antioxidant activity; cyclic voltammetry**

## 1. Introduction

Natural antioxidants seem to play a very important role in weakening the concentration of reactive oxygen and nitrogen species, which are maleficent and highly reactive intermediates constantly produced due to numerous biological reactions (Fernandez-Panchon, Villano, Troncoso, & Garcia-Parrilla, 2008; Señorans, Ibáñez, & Cifuentes, 2003). In this path, the studies on “discovering natural antioxidants” have become a subject of great interest in many areas, such as food chemistry, natural pharmaceutical products, and health care. Potential sources of antioxidant compounds derived from plants, chiefly phenolics, and medicinal activities have been searched in several types of plant materials (Belboukhari, Cheriti, & Belboukhari, 2013; Keffous et al., 2016; Spiridon et al., 2011). *Limoniastrum feei*, belonging to the Plumbaginaceae family, endemic of the Mediterranean bioclimatic Sahara stage (Djellouli & Daget, 1987). It is a low shrub (10–40 cm), with long and flat leaves in basal rosettes (Ozenda, 2004). As per traditional knowledge, it is used in the treatment of a variety of disorders, bronchitis, and stomach infections (Belboukhari & Cheriti, 2009), *Limoniastrum feei* leaves contained a potential biological activity, antifungal, antibacterial (Belboukhari & Cheriti, 2005), high quantities of phenolic compounds and present a potential antioxidant activity (Chaabi, Beghidja, Benayache, & Lobstein, 2008; El-Haci, Atik-Bekkara, Didi, Gherib, & Didi, 2012). In the last decade, the determination of antioxidant capacity and the total content of secondary metabolites from plants have been in wide demand. For this, the basic aim of this research is to quantify the total phenolics, total flavonoids (TF) content, and antioxidant activity of *Limoniastrum feei* aqueous extract. The antioxidant activity of extract has been evaluated using an *in vitro* model system, by different chemical and electrochemical methods, such as reducing power, DPPH radical scavenging activity, total antioxidant capacity (TAC) assay, cyclic voltammetry (CV), and superoxide scavenging assay.

## 2. Results and discussion

### 2.1. Extraction yield, total phenolic and flavonoids compounds

A phenolics compound including flavonoids is known as reliable and non-toxic antioxidants. Many experimental studies have shown that a high dietary intake of natural phenolic compounds is strongly associated with a longer life expectancy, reduced risk of various types of cancer, specific chronic diseases, diabetes, obesity, improved endothelial function, and reduced blood pressure (Halliwell, 2007; Hodgson & Croft, 2006; Yan & Asmah, 2010). Eleven phenolic compounds are isolated from the bioactive extract of the aerial part of *Limoniastrum feei* and two major constituents are identified as glucosylated flavonoids (Rahmani, Belboukhari, & Cheriti, 2014).

The amount of extraction yield (%) total phenolics content (TPC) and TF of *Limoniastrum feei* aqueous extract is shown in (Table 1).

The TPC was determined by Folin–Ciocalteu (FC) reagent in terms of gallic acid (GA) equivalents used the regression equation of the calibration curve obtained from GA ( $y = 0.0081x + 0.0852$ ,  $R^2 = 0.9974$ ). The results showed that the aqueous extract of *Limoniastrum feei* possesses the highest levels of TPC ( $200.28 \pm 2.75$  µg/mg) In general, the higher TPC resulted in higher antioxidant activity.

**Table 1. TF, total phenolic contents and extraction yield of *Limoniastrum feei* aqueous extract**

Yield (%)	Total phenolics(µg GA equivalents/mg dried extract)	TF (µg Quercetin equivalents/mg dried extract)
22	200.28 ± 2.75	54.77 ± 3.21

The concentration of flavonoids in *Limoniastrum feei* aqueous extract was determined using the spectrophotometric method with aluminum chloride ( $\text{AlCl}_3$ ). The content of flavonoids was expressed in terms of Quercetin equivalents (the standard curve equation:  $y = 0.034x + 0.0475$ ,  $R^2 = 0.9979$ ).

This confirms the assertion that phenolic contents of plants contribute directly to their antioxidant properties.

### 2.2. Reducing power

In this test, the presence of reductants in an extract causes the reduction of  $\text{Fe}^{3+}$ / ferricyanide complex to  $\text{Fe}^{2+}$ /ferrous according to the chemical reaction (Eq. 1)



The reducing power of the extract is expressed as Ascorbic acid equivalents ( $\mu\text{g}$  Ascorbic acid/mg sample).

$\text{Fe}^{3+}$  reduction to  $\text{Fe}^{2+}$  is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action (Yıldırım, Mavi, & Kara, 2001).

The reducing power of a compound is related to its electron transfer ability and may therefore serve as a significant indicator of its potential antioxidant activity (Meir, Kanner, Akiri, & Philosoph-Hadas, 1995).

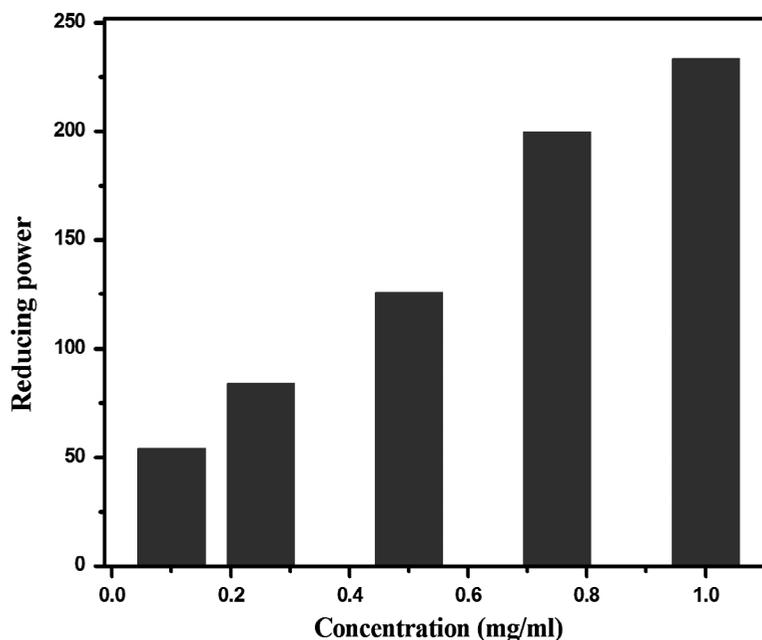
The reducing antioxidant power of *Limoniastrum feei* aqueous extract was expressed in terms of Ascorbic acid equivalents. The sample has a capacity that is equivalent to  $233.39 \pm 4.23 \mu\text{g}$  for milligram extract.

The reducing powers of water extract increase as the concentration of extract increases (Figure 1).

### 2.3. DPPH assay

Phenols are very important secondary metabolite plant constituents because of their scavenging capacity on free radicals due to their hydroxyl groups. Therefore, the phenolic contents of plants may contribute directly to their antioxidant action (Tosun et al., 2009).

Figure 1. Reducing power from *Limoniastrum feei* aqueous extract expressed in micrograms of Ascorbic acid equivalents per milligram extract.



DPPH<sup>•</sup> radical scavenging activity is widely used as an index to evaluate the antioxidant activity of medicinal plants. Substances which can change the DPPH<sup>•</sup> free radical color from violet to yellow upon reduction by either the process of electron- or hydrogen donation can be considered as antioxidants and therefore radical scavengers (Brand-Williams, Cuvelier, & Berset, 1995).

The examination of antioxidant activity of *Limoniastrum feei* aqueous extract showed different values (Figure 2). The obtained values varied from  $23 \pm 1.6$  to  $89.11 \pm 2.3\%$ .

The DPPH<sup>•</sup> radical elimination activities values depending on the dose of *Limoniastrum feei* aqueous extract.

The value of IC<sub>50</sub> in mg/ml expresses the effective concentration of the antioxidant extract necessary for trapping 50% of DPPH radicals dissolved in methanol, according to the results achieved; the aqueous extract has a good antioxidant activity value of  $0.58 \pm 0.03$  mg/ml.

#### 2.4. Total antioxidant capacity (TAC) assay

The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. In addition, they have a metal chelation potential (Rice-evans, Miller, Bolwell, Bramley, & Pridham, 1995).

The TAC is based on the reduction of molybdenum hexavalent oxidation state Mo(VI) to molybdenum pentavalent Mo(V) by the effect of the electron donor by the antioxidant and formation of molybdenum complex colored green to acid pH, according to the chemical reaction (Eq. 2).

The Mo(VI) to Mo(V) reducing activity is expressed as Ascorbic acid equivalents ( $\mu\text{g}$  Ascorbic acid per milligram sample).

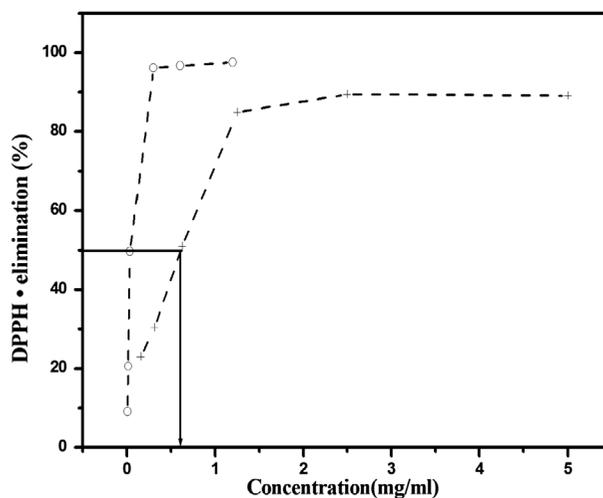


The *Limoniastrum feei* aqueous extract showed a significant activity in this test of the order  $112.4 \pm 1.97$   $\mu\text{g}$  of Ascorbic acid equivalents for one milligram of aqueous extract.

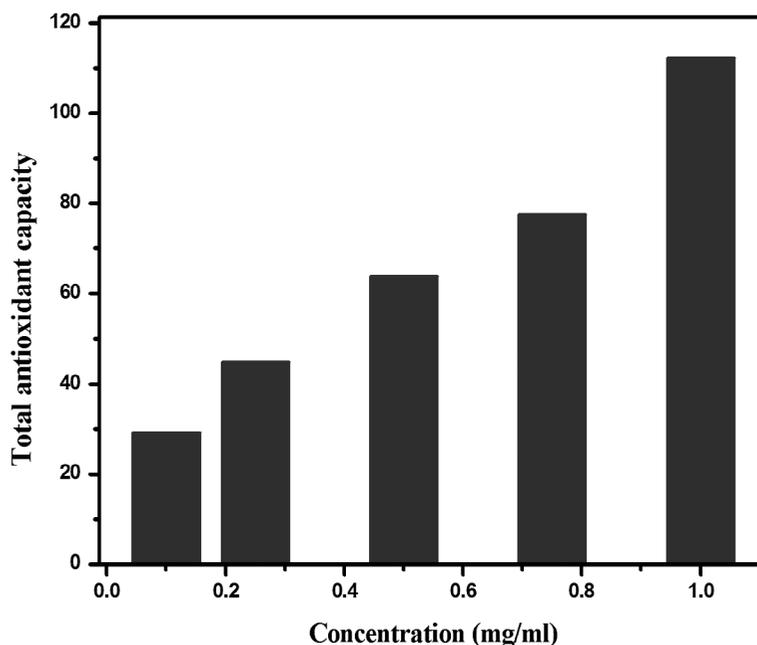
The TAC of aqueous extract increases as the concentration of extract increases (Figure 3). The samples show different antioxidative values depending on the concentration.

The high contents of phenolic compounds indicated that these compounds contribute to the antioxidant activity.

Figure 2. DPPH<sup>•</sup> scavenging activity (%) of *Limoniastrum feei* aqueous extract compared to Quercetin.



**Figure 3. TAC of *Limoniastrum feei* aqueous extract expressed as micrograms of ascorbic acid equivalents per milligram extract.**



### 2.5. Cyclic voltammetry

CV method is frequently preferred for various types of physicochemical analysis in redox system due to their high sensitivity, rapidity, simplicity, the possibility of performing analysis in colored or turbid solutions as well as the portability of the instrumentation (David et al., 2015).

CV of Ascorbic acid in an aqueous solution containing KCl (0.1 M) as supporting electrolyte at pH 3.2 shows one irreversible anodic peak at 0.36 V versus Ag/AgCl reference electrode (Figure 4 (b)) that corresponds to the oxidation of ascorbic acid to dehydro-ascorbic acid which is unstable and degrades, as no corresponding reduction peak appears (Litos, Aletras, Hatzipanayioti, Kamariotaki, & Lymberopoulou-Karaliota, 2007).

The absence of the corresponding reduction peak reflects the irreversibility of dehydroascorbic acid in the electrode reaction, as confirmed earlier by other authors (Pisoschi, Pop, Negulescu, & Pisoschi, 2011; Sun-Waterhouse, Smith, O'Connor, & Melton, 2008).

Such low oxidation potential and pronounced electron donating ability render Ascorbic acid a highly potent antioxidant with the most powerful reducing ability (Sun-Waterhouse et al., 2008).

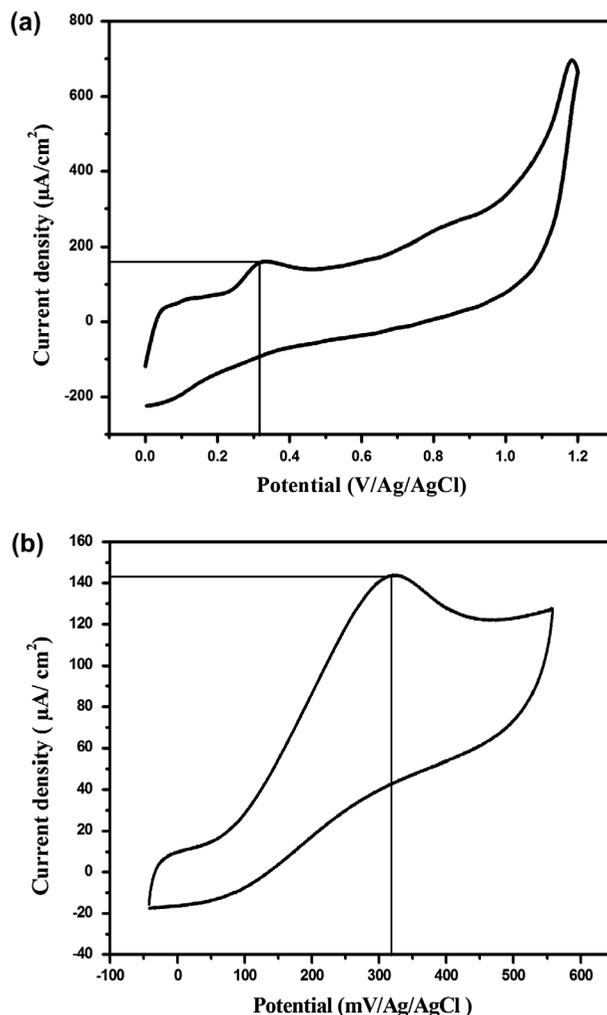
The voltammogram of the aqueous extract solution containing KCl (0.1 M) as a supporting electrolyte presented one anodic peak at approximately 300–320 mV versus Ag/AgCl (Figure 4 (a)). The absence of the corresponding reduction peak also points to the irreversibility of oxidation of reactional products produced in this reaction.

In general, compounds with a lower formal potential are better reducing agents and have higher antioxidant activity (Kilmartin, 2001; Sousa, da Rocha, Cardoso, Silva, & Zanoni, 2004) and good correlations have been observed between antioxidant properties and redox potentials (Firuzi, Lacanna, Petrucci, Marrosu, & Saso, 2005; Galato et al., 2001).

### 2.6. Superoxide scavenging assay

The electrochemical behavior of oxygen in the presence of an antioxidant substrate and these interaction mechanisms are studied by different authors (Korotkova, Karbainov, & Avramchik, 2003; Le Bourvellec, Hauchard, Darchen, Burgot, & Abasq, 2008). This test is applied for evaluated different

Figure 4. Cyclic voltammograms recorded for (a) *Limoniastrum feei* aqueous extract and (b) ascorbic acid in KCl 0.1 mol L<sup>-1</sup> as a supporting electrolyte, at scan rate. 100 mVs<sup>-1</sup>.



antioxidant substrates: Phenolic compounds (René, Abasq, Hauchard, & Hapiot, 2010), extracts (Ahmed & Shakeel, 2012; Blanc, Hauchard, Audibert, & Gall, 2011; Ghiaba, Yousfi, Hadjadj, Saidi, & dakmouche, 2014), and vitamin E derivative, tocopherol (Korotkova, Avramchik, Kagiya, Karbainov, & Tcherdyntseva, 2004).

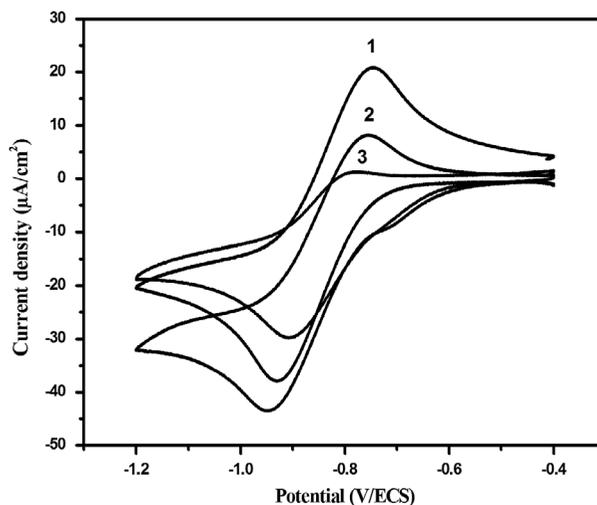
The O<sub>2</sub><sup>-</sup> radical scavenging activity obtained for the aqueous extracts of *Limoniastrum feei* is presented in (Figure 5). From the values obtained from voltammogram, the inhibition percentage was calculated using the formula given earlier. The standard antioxidant used is Ascorbic acid. It showed powerful anti-radical activity of the order 93% and the *Limoniastrum feei* aqueous extract showed average activity in this test of order 61.46 ± 2.51% at 0.5 mg/ml doses.

### 3. Experimental procedure

#### 3.1. Chemicals

FC reagent, GA, 1,1-diphenyl-2-picryl hydrazyl (DPPH) (Sigma), Quercetine, Sigma-Aldrich. Acide ascorbique (AA) (cheminova), sulfuric acid (H<sub>2</sub>SO<sub>4</sub>), ferrous chloride (FeCl<sub>3</sub>), AlCl<sub>3</sub>, sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) (Biochem), potassium ferricyanide K<sub>3</sub>Fe(CN)<sub>6</sub> (FLUKA), trichloroacetic acid (TCA) (Prolabo), anhydrous sodium phosphate, and ammonium molybdate), methanol.

**Figure 5. Cyclic voltammograms of O<sub>2</sub> reduction in the absence of antioxidant (1), in the presence of *Limoniastrum fei* aqueous extract (2) and ascorbic acid (3) at a steady glassy carbon disk electrode in DMF/0.1 M Bu<sub>4</sub>NPF<sub>6</sub>. Scan rate 50 mVs<sup>-1</sup>.**



### 3.2. Plant collection

*Limoniastrum fei* species was selected based on their beneficial uses in traditional medication. Aerial parts of *Limoniastrum fei* were collected from Lahmar district (southwest of Algeria) in February 2012.

### 3.3. Preparation of extract

Twenty grams of the aerial part (stems and leaves) of *Limoniastrum fei* was extracted by 400 ml of distilled water for 3 h under reflux. The extracts were then filtered and concentrated under reduced pressure at 70°C using a rotary evaporator (Büchi Rotavapor R-210) to obtain the *Limoniastrum fei* crude extract. The last one was kept in dark and stored at room temperature (Majhenič, Škerget, & Knez, 2007).

### 3.4. Determination of total phenolic content (TPC)

The FA assay, adapted from Singleton and Rossi (1965), was used for the determination of total phenolics present in the *Limoniastrum fei* aqueous extract.

A volume of 200 ml of the extract was mixed with 1 ml of FC reagent diluted 10 times with water and 0.8 ml of a 7.5% sodium carbonate aqueous solution in a test tube. The tube was mixed and allowed to stand for 30 min. The absorbance was measured at 765 nm using a SPECORD®200 plus UV-Visible spectrophotometer.

TPC was expressed as gallic acid equivalents (GAE) in micrograms per milligram dry material. Using the following equation based on the calibration curve:  $y = 0.0081x + 0.0852$ ,  $R^2 = 0.9974$  where  $x$  was the absorbance and  $y$  was the GAE ( $\mu\text{g}/\text{mg}$ ). The data reported three replications.

### 3.5. Total flavonoids content (TFC)

The amounts of TF in *Limoniastrum fei* extract were determined according to the Bahorun et al. (1996) procedure. To 1 ml of extract switch in a test tube, 1 ml of methanol solution of AlCl<sub>3</sub> to 2% was added and incubated for 15 min at room temperature. Absorbance was read from the UV-Visible spectrophotometer (SPECORD®200 plus) at 430 nm.

TF contents were calculated from the calibration curve of Quercetin standard solutions, and expressed as  $\mu\text{g}$  Quercetin equivalent/mg dry material, using the following equation:  $y = 0.034x + 0.0475$ ,  $R^2 = 0.9979$ , where  $x$  was the absorbance and  $y$  was the Quercetin equivalents ( $\mu\text{g}/\text{mg}$ ).

### 3.6. Antioxidant capacity determinations

#### 3.6.1. Reducing power

The reducing antioxidant power of *Limoniastrum feei* aqueous extract was determined according to the method described by Oyaizu (1986). To 2.5 ml of different concentrations (0.25–1 mg/ml) of *Limoniastrum feei* extract were added, 2.5 ml of 0.2 M phosphate buffer (pH 6.6), and 2.5 ml of 1% aqueous potassium hexacyanoferrate  $K_3Fe(CN)_6$  solution. After 20 min of incubation at 50°C, 2.5 ml of 10% TCA was added to the mixture and centrifuged at 3000 rpm for 10 min.

The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and 0.5 ml of 0.1%  $FeCl_3$ . The absorbance was measured spectrophotometrically at 700 nm. Reducing power activity was expressed as Ascorbic acid equivalents per milligram extract). A blank solution was prepared without adding sample. The values are presented as the means of triplicate analysis.

#### 3.6.2. Scavenging effects on DPPH radicals

The DPPH assay was done by measuring the decrease in absorbance of methanolic DPPH solution at 515 nm in the presence of different concentrations (0.1–1.25 mg/ml) of plant extract according to the method described by Brand-Williams et al. (1995). With some modifications, a mixture of 50  $\mu$ l plant extract and 1250  $\mu$ l DPPH (2 mg% in methanol) was incubated for 30 min and the absorbance read at 515 nm.

The results were expressed in percentage as a DPPH• elimination (I%).

$$I\% = [(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100$$

I% is the percentages as a DPPH• elimination.

$Abs_{control}$  is the absorbance value of the DPPH solution without samples.

$Abs_{sample}$  is the absorbance value of the DPPH solution with samples.

The  $EC_{50}$  value was determined graphically by linear regression.

#### 3.6.3. Total antioxidant capacity (TAC) assay

The assay was based on the reduction of Mo (VI) to Mo (V) by the samples and subsequent formation of a green phosphate/Mo (V) complex at an acid pH, according to the method of Prieto, Pineda, and Aguilar (1999). The aqueous extract of *Limoniastrum feei* (0.3 ml) different concentrations (0.25–1 mg/ml) was combined with 3 ml of reagent solution (0.6 M  $H_2SO_4$ ,  $28 \times 10^{-3}$  M sodium phosphate and  $4 \times 10^{-3}$  M ammonium molybdate). The mixture was incubated for 90 min at 95°C. After cooling to room temperature, the absorbance of the solution was measured at 695 nm against a blank. The total antioxidant activity of *Limoniastrum feei* aqueous extract was expressed in terms of Ascorbic acid equivalents per milligram extract.

#### 3.6.4. Cyclic voltammetry

The electrochemical methods have been broadly used to investigate the redox properties of natural phenolics (Born et al., 1996; Simić, Manojlović, Šegan, & Todorović, 2007). Voltammetry is a convenient methodology for the study of antioxidant properties and the determination of the antioxidant activity of biological systems (Arulpriya, Lalitha, & Hemalatha, 2010).

Voltalab PGZ 301 made up of a potentiostat-galvanostat equipped with Volta Master 4 software was used for voltammetric experiments. A two-compartment Pyrex cell using a conventional three-electrode configuration was used to perform Cyclic voltammograms experiments, a glassy carbon disk working electrode (GCE), 2 mm in diameter, polished before each measurement, a platinum wire

counter electrode, and an Ag/AgCl saturated KCl reference electrode were used. Glassy carbon is thus an excellent electrode material to study the electrochemistry of natural antioxidants (Kilmartin, 2001).

About 15 ml of supporting solution was dispensed into an electrochemical cell. To it was added appropriate volume of plant extracts. Cyclic voltammograms measurements were performed at positive potentials, at a scan rate of 100 mVs<sup>-1</sup> at room temperature at pH ~3.2 in the presence of KCl (0.1 M) as a supporting electrolyte.

### 3.6.5. Superoxide scavenging assay

The superoxide anion radical scavenging assay was performed according to the original method reported by E.I. Korotkova, Karbainov, and Shevchuk (2002) with some modifications. The CV technique was used to generate the superoxide radical O<sub>2</sub><sup>•-</sup> by the one-electron reduction of oxygen molecular saturated in DMF media. A conventional three-electrode cell was used with: (a) a saturated calomel electrode reference electrode, (b) a platinum wire counter electrode, and (c) a 3-mm diameter glassy carbon working electrode.

A solution of 5 ml of DMF containing the supporting electrolyte 0.1 M Bu<sub>4</sub>NPF<sub>6</sub> was saturated by molecular oxygen. The cyclic voltammogram measurements (CV) of the oxygen reduction were run from 0 mV to + 1200 mV at a scan rate of 50 mVs<sup>-1</sup> at room temperature and the superoxide anion radical scavenging was assessed from the change in the cathodic current of the voltammograms in the absence and presence of antioxidant substrate. The results were expressed as percent inhibition (I %) and were calculated by the following formula, Ascorbic acid was used as a standard.

$$I\% = [(I_{pa}^0 - I_{pa}^s) / I_{pa}^0] \times 100$$

I% = percent inhibition of superoxide radical O<sub>2</sub><sup>•-</sup>

I<sub>pa</sub><sup>0</sup> = the anodic peak current of O<sub>2</sub><sup>•-</sup> oxidation without sample

I<sub>pa</sub><sup>s</sup> = the anodic peak current of O<sub>2</sub><sup>•-</sup> oxidation with sample

## 4. Descriptive statistics

MICROSOFT EXCEL® (USA) was used for statistical analyses.

Results are expressed as mean ± S.D. unless noted otherwise.

## 5. Conclusions

The analytical investigations reveal that aqueous extract of aerial part of *Limoniastrum feei* showed the highest phenolics compounds. In addition, the presence of flavonoids in *Limoniastrum feei* (Belboukhari & Cheriti, 2007; Rahmani et al., 2014) may be responsible for the potent antioxidant and antiradical-scavenging activity evaluated by different chemical and electrochemical methods, such as reducing power, DPPH radical scavenging activity, TAC assay, and CV. In all the tests that have been performed demonstrate that the antioxidant capacity values were depending on the concentration of *Limoniastrum feei* aqueous extract. Therefore, *Limoniastrum feei* extract could serve as valuable source of natural antioxidants in both pharmaceutical and food industries.

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