IN VITRO ANTIOXIDANT ACTIVITY AND TOTAL PHENOLIC CONTENTS IN METHANOL CRUDE EXTRACTS FROM THE ALGERIAN MEDICINAL PLANT *LIMONIASTRUM FEEI*

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Abstract: The use of synthesis antioxidant molecules is being dangerous anyway, because of the potential toxicological risks. In fact, new source of natural antioxidant molecules are required. The phenolics, natural compounds largely present in the vegetable kingdom, are recognized for their antioxidant activity and still little exploited by different industry. Our work aim is to evaluate the antioxidant capacity of the phenolic compounds extracted from the Algerian medicinal plant: *Limoniastrum feei*. This plant presented high levels of polyphenols ranged from 232.69 to 254.90 mg gallic acid equivalents/g dried extract, and flavonoids ranged from 106.33 to 129.66 mg catechin equivalents/g dried extract. The antioxidant activity of the methanolic crude extracts were evaluated using different antioxidant assays, including: reducing power and DPPH free radical scavenging activity. The results showed that the methanolic extract of *Limoniastrum feei* presented an average reducing power and more potent inhibition of

DPPH radical activity. The study of organic fractions of this plant may reveal interesting results.

Keywords: *Limoniastrum feei, phenolic compounds, flavonoids, antioxidant activity, reducing power, DPPH*

INTRODUCTION

The oxidative damage caused by reactive oxygen species (ROS) such as the super oxide radical ($O_2^{\bullet -}$) and by hydroxyl radicals ($HO^{\bullet}$) on lipids, proteins, and nucleic acids may trigger various diseases including cancer, cardiovascular diseases, cataracts, atherosclerosis, diabetes, immune deficiency diseases and ageing [1 – 3]. Ascorbic acid, α-tocopherol and phenolic compounds, which are present naturally in vegetables, fruits, grains and pulses, posses the ability to reduce the oxidative damage [2]. Synthetic antioxidants such as BHT (butylated hydroxytoluene), BHA (butylated hydroxyanisol), and TBHQ (tertiary butylhydroquinone) have commonly been used to prevent oxidative deterioration of fats and oily foods [4]. Natural and synthetic antioxidants have been shown to enhance product stability, quality and shelf. Many research works have mentioned the disadvantage of synthetic antioxidants [4 – 7]. Indeed, the use of synthetic antioxidant in food products has decreased due to their instability, as well as their possible toxic and carcinogenic effects on health [5]. Therefore, research into the determination of natural antioxidant sources is important. The number of reports about isolation and testing of natural antioxidants has increased immensely during the last decade. In the search for sources of natural antioxidants, some medicinal plants have been extensively studied for their antioxidant activity and radical scavenging activity in the last years [8]. A great number of plants worldwide showed a strong antioxidant activity and a powerful scavenging activity against free radicals [9 – 10]. Nowadays, research has focused on medicinal plants to extract new natural antioxidants that can replace synthetic additives. In addition, the public's belief that phytochemicals are inherently safer than synthetic chemicals [11]. Phenolics are an important class of secondary plant metabolites possessing an umpressive array of pharmacological activity. One of the more prominent properties of the phenolics is their excellent radical scavenging ability [12]. This one is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, singlet oxygen quenchers agents, and they have also metal chelating potential [13]. One of the medicinal plants used for various medicinal purposes in Algerian folk medicine is *Limoniastrum feei* (Plumbaginaceae). The plant is native to southeast of Algeria, northern Africa [14]. *L. feei* is used to treat gastric infections, bronchitis and stomach infection [15].
The aim of present works is to study *in vitro* antioxidant activities of the methanolic extracts of *L. feei*, using ferric reducing antioxidant power (FRAP) and DPPH radical scavenging assays. In addition, the total content of phenolics and flavonoids from plant extracts were also measured.

MATERIAL AND METHODS

Chemicals

Butylated hydroxyanisol (BHA), Butylated hydroxytoluene (BHT), 1,1-diphenyl-2-picryl-hydrazyl (DPPH), potassium ferricyanide [$K_3Fe(CN)_6$], trichloroacetic acid (TCA), Folin-Ciocalteu's phenol reagent and ascorbic acid were purchased from Fluka (Switzerland). Sodium carbonate and sodium hydroxide were from Merck (Germany). $FeCl_3$ was from Sigma Chemical Co (Germany). Methanol was from Biochem (Chemopharma, UK). All other chemicals were of analytical grade.

Plant materials

The whole plant of *Limoniastrum feei* were collected in December 2007 from Aïn Ouarka (Region of Naâma, south-west of Algeria) and dried away from direct sunlight. The plant was identified at the laboratory of Ecology and Management of Natural Ecosystems of the University of Tlemcen (Algeria). Dried plant material was then crushed into a mortar and stored at very low temperature until further use.

Sample preparation

A powder (20 g) of each part of the plant (leaves and stems) was extracted by 100 mL of methanol–water (7:3, v/v) at 80 °C for 3 h under reflux [16]. The extracts were then filtered and concentrated under reduced pressure at 60 °C using a rotary evaporator (Büchi Rotavapor R-200) to obtain the methanolic crude extract. The last one was kept in dark and stored at 4 °C.

Total phenolics content

Total phenolics were estimated by the Folin-Ciocalteu method [17]. 0.1 mL of the methanolic crude extract was mixed with 2 mL of sodium carbonate (2 %) freshly prepared, the whole was vigorously mixed on a vortex. After 5 min, 100 µL of Folin-Ciocalteu reagent (1N) were added to the mixture, all was left for 30 min at room temperature and the reading is performed against a blank at 750 nm. A calibration curve was performed in parallel under the same operating conditions using gallic acid as a positive control. The results are expressed as mg gallic acid equivalent per gram of dry extract (mg GAE/g).

Total flavonoids content

The total flavonoid content of each part of *L. feei* extract was determined by a colorimetric method as described in the literature [18]. Each sample (0.5 mL) was mixed with 2 mL of distilled water and subsequently with 0.15 mL of a $NaNO_2$ solution (15 %). After 6 min, 0.15 mL of aluminum chloride ($AlCl_3$) solution (10 %) was added and allowed to stand for 6 min, then 2 mL of NaOH solution (4 %) was added to the mixture. Immediately, water was added to bring the final volume to 5 mL and the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was then determined at 510 nm versus prepared water blank. Results were expressed as catechin equivalent (mg catechin/g dry extract).

Ferric reducing antioxidant power assay

The reducing power of the different part of *L. feei* was determined according to the method of Yang et al. [19]. The methanolic crude extracts, BHA and BHT were used at different concentrations (0.1, 0.2, 0.3, 0.4, 0.5 mg.mL^{-1}). One milliliter of each sample was mixed with phosphate buffer (2.5 mL, 0.2 mol.L^{-1}, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 30 mmol.L^{-1}). The mixture was incubated at 50 °C for 20 min. A 2.5 mL TCA (0.6 mol.L^{-1}) was added to the mixture, which was then centrifuged for 10 min at 3000 g. The supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 6 mmol.L^{-1}), and the absorbance was measured at 700 nm in a spectrophotometer (Jenway 6400).

Determination of the scavenging effect on DPPH radicals

A methanolic solution (50 µL) of each crude extracts at different concentrations was added to 1.95 mL of DPPH solution (6×10^{-5} M in methanol) [20]. The studied compounds were tested with methanol as control, BHA and BHT as antioxidant references and absorbance at 515 nm was determined after 30 min. The absorbance (*A*) of the control and samples was measured, and the DPPH scavenging activity (*SA*) in percentage was determined as follow:

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

IC_{50} was obtained graphically from nonlinear regression analysis.

Statistical analysis

Data are presented as the mean ± standard deviation (SD) of each triplicate test.

RESULTS AND DISCUSSION

Total phenolic and flavonoid content

Determination of total phenolic and flavonoid contents of the methanolic crude extracts of *L. feei* were done by using Folin–Ciocalteu colorimetric and $AlCl_3$ methods, respectively. Total polyphenol contents were estimated with Folin–Ciocalteu colorimetric method. This reagent is a mixture of phosphotungstic ($H_3PW_{12}O_{40}$) and phosphomolybdic acids ($H_3PMo_{12}O_{40}$). It is reduced during the oxidation of phenols in a mixture of blue oxides of tungsten and molybdenum. The color produced, whose absorption maximum is between 700 and 750 nm, is proportional to the amount of polyphenols present in plant extracts. The total phenolic contents were reported as mg gallic acid equivalent per gram of dry extract. The methanolic crude extract of *L. feei* shown a high phenolic compounds in the two extracts of the plant (leaves and stems), indeed, the methanolic crude extract of leaves contained a high phenolic compounds compared to the crude extract of the stems, as presented in Table 1. In $AlCl_3$ colorimetric method, aluminum chloride forms acid stable complex with the keto and/or the hydroxyl groups in the A or C ring of flavonoids [18]. The total flavonoid content was reported as mg catechin equivalent per g dried extract. The results, as presented in Table 1, show that the methanolic crude extract of leaves of *L. feei* contained high

flavonoids compounds compared to the crude extract of the stems. On this plant, there are very few publications that are made regarding the levels of polyphenols and flavonoids. Indeed, recently, the publication by Chaabi et al. [21] reports that *L. feei* contains seven polyphenolic constituents: gallic acid, myrciaphenone A, myricetin-3-*O*-*β*-galactopyranoside, epigallocatechin gallate, myricetin 3-*O*-*α*-rhamnopyranoside, quercetin and myricetin. The phytochemistry investigation of the water-acetone extract of twig part of *L. feei* led to isolation of four flavonoids. The structures of these compounds were identified as: 6,3',4'-tri-methoxy 3,5,5'-trihydroxy flavonol, 3-(6''-malonyl 2''-ramnosyl glucosil) 6,3',4'-trimethoxy 5,5'-dihydroxy flavonol, tetraacetate 7-dihydroxy-4'-methoxy 8-O-β-glucopyranoside isoflavone and tetraacetate 7,4'dimethoxy 8-O-β-glucopyranoside isoflavone [15]. This plant contains large amounts of phenolics (phenolic acids, tannins, flavonoids, flavonols, isoflavone) reflected by the high levels of this chemicals found in this study concerning phenolics and flavonoids.

Table 1. *Total phenolic[a] and total flavonoid[b] of methanolic crude extracts of L. feei*

		Total phenolic	Total flavonoid
Limoniastrum feei	Leaves	254.90 ± 22.88	129.33 ± 1.15
	Stems	232.69 ± 13.21	106.33 ± 4.93

Each value represents the mean ± SD (n = 3).
[a] Total phenolic content was expressed as mg gallic acid equivalents/g dried extract
[b] Total flavonoid content was expressed as mg catechin equivalents/g dried extract

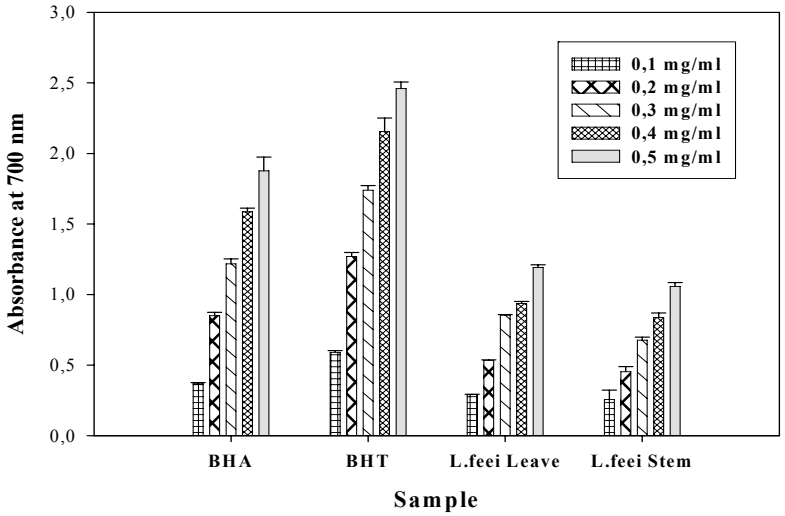

Figure 1*. Reducing power activity of methanolic crude extracts of L. feei*

Ferric reducing antioxidant power assay

In this assay, the antioxidant activity of samples was measured by their ability to reduce the Fe^{3+}/ferricyanide complex by forming ferrous products. Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue coloration at 700 nm. Increased absorbance at this wavelength indicates stronger reducing power. Figure 1 shows the reductive capability of methanolic extracts of the two parts of *L. feei* compared to BHA and BHT as standards. The reducing power of this extracts was in a concentration-

dependent manner. At all the concentrations, the antioxidant activity of the extracts were average compared to controls used (BHA and BHT). Nevertheless, both extracts have expressed a reductive, these results suggest that these samples have a potency to donate electron to reactive free radicals, converting them into more-stable metabolites and terminating the free radical chain reaction [19]. These results are considered with a critical eye. The activity of an extract or a fraction due to the intrinsic activity of the active components and their relative abundance, a negative result may be a consequence of the presence of a small amount of active ingredients in the extract as a large quantity of non-active compounds.

DPPH radical scavenging

The DPPH is a stable organic free radical with an absorption maximum band around 515-528 nm and it is a useful reagent for evaluation of antioxidant activity of compounds. The methanolic extracts of *L. feei* were tested for their antioxidant scavenging effects on DPPH radical and their activity was compared to the synthetic antioxidants BHA and BHT used as antioxidant references. The results obtained at different concentrations are given in Figure 2. From these results, it is demonstrated that all the tested extracts showed a non-linear dose-dependant activity. The free radical scavenging activity is also expressed by the antioxidant concentration required for a 50% DPPH reduction (IC_{50}) (Table 2).

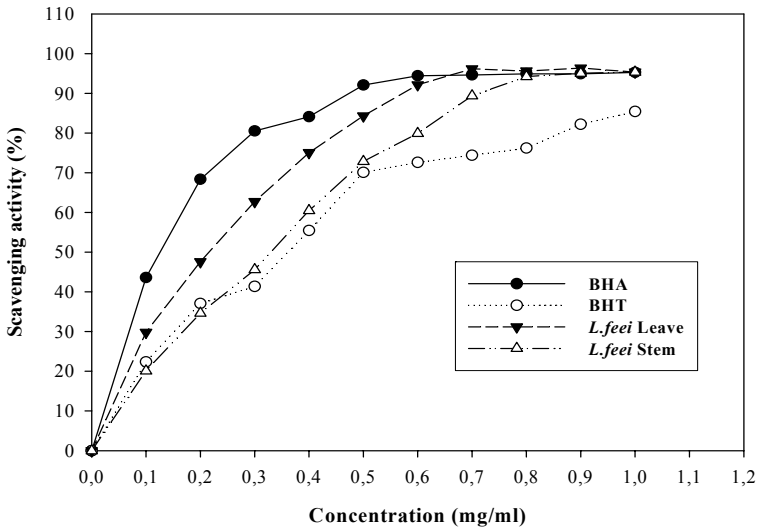

***Figure 2**. Scavenging activity of methanolic crude extracts of L. feei on the DPPH radical*

***Table 2**. The IC_{50} value[a] of methanolic crude extracts of L.feei, BHA and BHT*

Limoniastrum feei	**Leaves**	4.75 ± 0.06
	Stems	7.07 ± 0.35
BHA		2.38 ± 0.11
BHT		7.53 ± 0.34

Each value represents the mean ± SD (n = 3)
[a] IC_{50} values were expressed as $\mu g.mL^{-1}$ (final concentrations)

The model for scavenging stable DPPH free radicals can be used to evaluate the antioxidative activities in a relatively short time. The absorbance decreases as a result of a color change from purple to yellow as the radical is scavenged by antioxidants through donation of hydrogen to form the stable DPPH-H molecule [22]. The effect of antioxidants on DPPH radical-scavenging was thought to be due to their hydrogen-donating ability. The preparations were able to reduce the stable free radical, DPPH, to the yellow-colored 1,1-diphenyl-2-picrylhydrazyl. The best free radical scavenging activities were exerted by the methanolic extract of leaves (IC_{50} = 4.75 $\mu g.mL^{-1}$) and stems (IC_{50} = 7.07 $\mu g.mL^{-1}$). As reference, the IC_{50} values of BHA and BHT were 2.38 and 7.53 $\mu g.mL^{-1}$, respectively. These results suggested that the methanolic extracts of *L. feei* are potent free radical scavengers.

CONCLUSION

In this work, we studied the antioxidant activity of methanolic extracts of stems and leaves parts of *L. feei* using two methods: the reduction power and free radical scavenging of DPPH. The results obtained show that this plant contains high enough levels phenolic and flavonoids compounds, this is consistent with the study of Chaabi et al. [21]. *L. feei* also showed remarkable antioxidant activity towards the reduction of iron, and a relatively high power against scavenging of free radicals. The studies of organic fractions of the plant to isolate the compounds that have good antioxidant properties, and identify compounds from each fraction are the prospects for this work.

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