Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(7):41-47

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Evaluation of antioxidants in ethanol extracts of *Limoniastrum guyonianum* (Zeïta) in Sahara of Algeria

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ABSTRACT

The objective of the present study was to evaluate the in vitro antioxidant activity of the ethanolic extract of Limoniastrum guyonianum from Algeria. The plant was evaluated using the free radical scavenging activity of the 1.1-diphenyl-2-picrylhydrazyl radical (DPPH), reducing power assay and Phosphomolybdenum method. Total phenolic content (TPC) was measured using a Folin–Ciocalteu assay. Total flavonoid content (TFC) was measured by aluminum chloride colorimetric assay. Phenolic compounds was found in the L. guyonianum of El Oued region (LE)(south of Algeria) from (11.39 to 91.51 mg GAE/100 g DW) and L. guyonianum of Ouargla region (LO)(south of Algeria)from (15.51 to 75.81mg GAE/100 g DW). Effective scavenging Concentration (IC_{50}) On DPPH radical ranged from (0.11 to 0.16 mg/l) in LO, and ranged from (0.18 to0.25 mg/l) in LE. The AEAC values of the L. guyonianum ranged from (1.8 to 2.16mM) in LO, and ranged from (0.55 to 2.14mM) in LE. The Phosphomolybdate antioxidant activity of the plant extracts ranged from (1.25 ± 0.07 to 7.94 ± 0.06 mM).

Key words: *Limoniastrum guyonianum*, Phytochemical Screening, Phenolics compounds, Flavonoid compound, Antioxidant activity, DPPH, Reducing power, Phosphomolybdate.

INTRODUCTION

The commercial development of plants as sources of antioxidants to enhance health and food preservation is of current interest. Epidemiological studies have suggested positive associations between the consumption of phenolic-rich foods or beverages and the prevention of diseases. These effects have been attributed to antioxidant components such as plant phenolics, including flavonoids [1].Herbal medicines are an important part of the culture and traditions of African people. Today, most of the population in urban South Africa, as well as smaller rural communities, is reliant on herbal medicines for their health care needs. Apart from their cultural significance, this is because herbal medicines are generally more accessible and affordable [2].*Limoniastrum guyonianum (*Zeïta*)* is a plant covered with calcareous concretions of 20 to 40 cm height, having erect branches, linear and semi-cylindrical leaves of 30 to 50mm, the sessiles are surrounding the stem Figure1[3].this endemic species belongs to Plumbaginaceae [4]. Has been used in traditional medicines to treat gastric infections. It has also been employed as an anti-bacterial drug in the treatment of bronchitis [5].Free radical induced oxidative damage is involved in the pathogenesis of many chronic and degenerative diseases, [6] such as cardiovascular disease [7, 8] cancer, diabetes [9] AIDS and infertility [10].These natural antioxidants or other compounds that can neutralize free radicals may be of central importance in the prevention of vascular diseases, some forms of cancer [11].

The present study deals for the first time the comparison of phytochemical screening, total phenolic content and Antioxidant activity of two *Limoniastrum guyonianum* (Zeïta) from Ouargla and El Oued regions in Saharan Algeria using Ethanol solvent.

Figure 1: ***Limoniastrum guyonianum***

EXPERIMENTAL SECTION

2.1. Plant material
*L.*guyonianum (Plumbaginaceae) was collected from the Ouargla region (south of Algeria), in February 2012. And from El Oued region (south of Algeria), in February 2011, plants have been drying in the shade and stored in paper bags.

2.2. Chemicals and reagents
All solvents and reagents used in the experiments were of the highest purity and purchased from Merck.Hydrochloricacid (HCl), Trichloroacetic acid ($FeCl_3$),Sodium hydroxide(NaOH), Sulfate copper($CuSO_4$), Chloroform ($CHCl_3$), Folin–Ciocalteu reagent, Sodium carbonate (Na_2CO_3), Aluminum chloride ($AlCl_3$), 2.2-dipheny l-1-picrylhydrazyl (DPPH) radical, Sodium phosphate (Na_2HPO_2),Sodium dihydrogen phosphate (NaH_2PO_4),Gallic acid ($C_7H_6O_5$).

2.3. Preparation of the extracts
Powdered aerial parts of *L. guyonianum* (10 g) were macerated with100 ml of petroleum ether,were extracted with 100 ml of ethanol: water (60: 40, v/v) for 24 h at room temperature the procedure was repeated twice. After filtration, the filtrate was evaporated, recovered with distilled water and partitioned successively using ethyl acetate and n-Butanol. The extraction phenolics were dissolved in methanol. Methanolic solutions of phenolic were kept frozen until analysis.

2.3. Determination of total phenolic content (TPC)
Total phenolic content of each extract was determined in duplicate by the Folin–Ciocalteu procedures [12].100µl of extract was transferred into a test tube and 1.5 ml of Folin-Ciocalteu reagent (previously diluted 10-fold with water) were added and mixed. The mixture was allowed to stand at room temperature for 5 min 1.5ml of (6% w/v) sodium carbonate was added to the mixture and then mixed gently. After standing at room temperature for 90 min, the absorbance was read at 725 nm using a UV-Vis spectrophotometer.

2.4. Determination of total flavonoid content (TFC)
The total flavonoid content (TFC) was determined according to the aluminum chloride colorimetric method [13].1 ml of diluted solution containing flavonoids and 1 ml of 5% (w/w) $NaNO_2$ were mixed for 6 min, and then 1 ml of 10% $AlCl_3$ (w/w) was added and mixed, 6 min later, 10 ml of 1 mol/l NaOH and incubated for 15 min in the obscurity room temperature was added. The absorbance of the solution was measured at 510 nm with UV-spectrophotometer.

2.5. Quantification of antioxidant activity
2.5.1. DPPH free-radical scavenging assay
The antioxidant capacity of the *L.guyonianum* extracts was measured using a DPPH method [14].For each sample, ten concentrations were tested in order to obtain their calibration curves, different concentrations of the extract150 µl were added to 3 ml 0.1 mM DPPH solution in methanol. After mixing vigorously the tubes were incubated in dark. After 30 min the absorbance was read at 517 nm. The inhibitory percentage of DPPH was calculated according to the following equation scavenging effect (%).

$$(\%) = [(A_0 - A_1)/A_0] \times 100$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the Sample.

2.5.2. Reducing power assay

The reducing power of the solvent extract was determined according to the method of Kumaran and Joel Karunakaran [15] with slight modification. Different amounts of each extracts in water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and Potassium ferricyanide $K_3Fe(CN)_6$ (2.5 ml, 1% w/v). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of Trichloroacetic acid (10% w/v) was added to the mixture. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1% w/v), and the absorbance was measured at 700 nm. The experiment was repeated for three times.

2.5.3. Phosphomolybdenum Assay (PM)

The antioxidant activity of the extract was evaluated by the Phosphomolybdenum method [16]. A 0.3 ml extract was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). In case of blank 0.3 ml of Water was used in place of extracts. The tubes containing the reaction solution were capped and incubated in a boiling water bath at 95°C for 90 min. After cooling to room temperature, the absorbance of the solution was measured at 695 nm using a spectrophotometer.

2.6. Statistical analysis

The results were presented as the mean values ± SD. All measurements were replicated three times, Correlation analysis between of antioxidant activities were carried out using the correlation and regression programmed in the EXCEL and Origin Pro8 program.

RESULTS AND DISCUSSION

3.1. Phytochemical screening

Phytochemical screening of the water extract was carried out to detect the presence of secondary metabolites such as flavonoids, tannins and steroids, using standard phytochemical methods [17-21]

Table 1: Phytochemical constituents of *L.guyonianum*

Chemical groups	*LO*	*LE*
Alkaloids	-	-
Phenols	+	+
Flavonoids	+	+
Saponins	-	+
Proteins	-	-
Tannins	+	+
Steroids	-	-
triterpenoids	-	-
Terpenoids	-	-
Coumarins	+	+
glycosides	-	-
reducing sugar	-	-

Note: '+' indicates presence and '-' absence

The results of phytochemical screening of *L.guyonianum* show that phenols, flavonoids, tannins and Coumarins are present of *LO* and *LE*. However, proteins, alkaloids, steroids, triterpenoids, terpenoids, glycosides and reducing sugar were not detected of *LO* and *LE*. However saponins are present in plant of *LE* but not detected in *LO* (Table 1).

3.2. Extraction yield

In Table 2 amount of yield varied widely in the *L. guyonianum* ranging from (0.609 %to 2.133%).*LO* butanol fraction had the highest yield estimated at (2.133%), and *LO* ethyl acetate fraction had the lowest estimated (0.609%).

3.3. Determination of total phenolic content (TPC)

Plant phenolics constitute one of the major groups of compounds acting as primary antioxidant free radical terminators [22].

As shown in Table 2 great variation in phenolic content was observed in *LO* and *LE*, ranging from (11.39 to 15.51 mg GAE/100 g DW) in ethyl acetate fraction and (75.81 to 91.51 mg GAE/100 g DW) in butanol fraction. *LE* butanol fraction had the highest phenolic content (91.51 ± 0.66 mg GAE/100 g), while the lowest was observed in

LE ethyl acetate fraction (11.39 ± 0.04 mg GAE/100 g)Table 2.The order of TPC in *L. guyonianum* butanol fraction is:*LE* > *LO* As for the ethyl acetate fraction is :*LO* > *LE*.

In our work, higher phenolic content was obtained in butanol fraction compared to ethyl acetate fraction as shown in Figure 2.

Table 2: Total phenolic content (TPC), total flavonoid content (TFC) of *L.guyonianum*

	Fraction	*LO*	*LE*
Yield (%)	Ethyl acetate	0.609	0.192
	Butanol	2.111	2.133
TPC (mg GAE/100 g DW)*	Ethyl acetate	15.51 ± 0.07	11.39 ± 0.04
	Butanol	75.81 ± 0.85	91.51± 0.66
TFC(mg QCE/100 g DW)*	Ethyl acetate	3.56 ± 0.02	2.85 ± 0.05
	Butanol	13.44 ± 0.33	10.43 ± 0.60

LO: L. guyonianum of Ouargla region LE: L. guyonianum of El Oued region
**Results are expressed as mean of 3 values ± standard deviation*

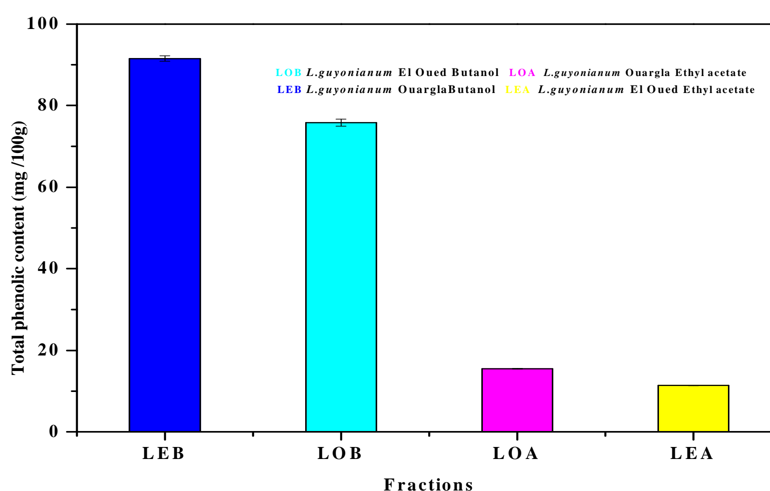

Figure 2: Total phenolic content of *L.guyonianum* (mg GAE/100 g DW)

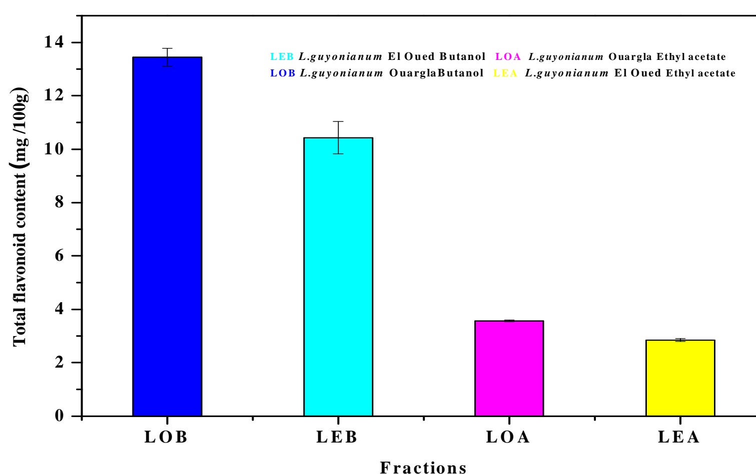

Figure 3: Total flavonoid content of *L.guyonianum* (mg QCE/100 g DW)

3.4. Determination of total flavonoid content (TFC)

Flavonoids are one of the most diverse and widespread group of natural compounds and are probably the most important natural phenolics. These compounds possess a broad spectrum of chemical and biological activities

including radical scavenging properties [23].The amount of TFC varied widely in the *L. guyonianum* ranging from (2.85 to 13.44 mg QCE /100 g DW),For to *LO* butanol fraction had the highest TFC estimated at (13.44± 0.33 mg QCE/100 g DW), and *LE* ethyl acetate fraction had the lowest estimated (2.85± 0.05 mg QCE/100 g DW) as shown in Table 2.

In our work, higher flavonoid content was obtained in butanol fraction compared to ethyl acetate fraction (Figure 3).

3.5. Quantification of antioxidant activity

3.5.1. DPPH free-radical scavenging assay

The DPPH free radical is a stable radical with a maximum absorption at 517 nm that can readily undergo scavenging by an antioxidant. So it has been widely accepted as a tool for evaluating the free radical scavenging activities of natural compounds [24].

Table 3 shows the IC50 values for the various fractions of *LE* and *LO* ranged from (0.11 mg/l to 0.25 mg/l). Highest value of IC50 (0.25 ± 0.03 mg/l) was detected in *LE* ethyl acetate fraction. Followed by *LE* butanol fractions (0.18 ± 0.01mg/l), while the lowest value of IC50 (0.11 ±0.01mg/l) was detected in *LO* ethyl acetate fraction and it corresponds to the highest antioxidant activity. The activity was in the order of butanol fraction is: *LO* > *LE* and the same for the ethyl acetate fraction.

Results of this research as shown in Figure4 showed that each the extracts did not exceed in its ability to the BHA, BHT and VC.

Table 3 Antioxidant activities in fractions of *L.guyonianum*

	Fraction	*LO*	*LE*
IC50 (mg/ml)*	Ethyl acetate	0.11 ± 0.01	0.25 ± 0.03
	Butanol	0.16 ± 0.01	0.18 ± 0.01
AEAC (mM)*	Ethyl acetate	2.16± 0.13	0.55 ± 0.04
	Butanol	1.89 ± 0.02	2.14 ± 0.03
EEAC(mM)*	Ethyl acetate	4.59 ± 0.3	1.25 ± 0.07
	Butanol	7.94 ± 0.06	6.27 ± 0.1

LO: L. guyonianum of Ouargla region LE: L. guyonianum of El Oued region
**Results are expressed as mean of 3 values ± standard deviation*

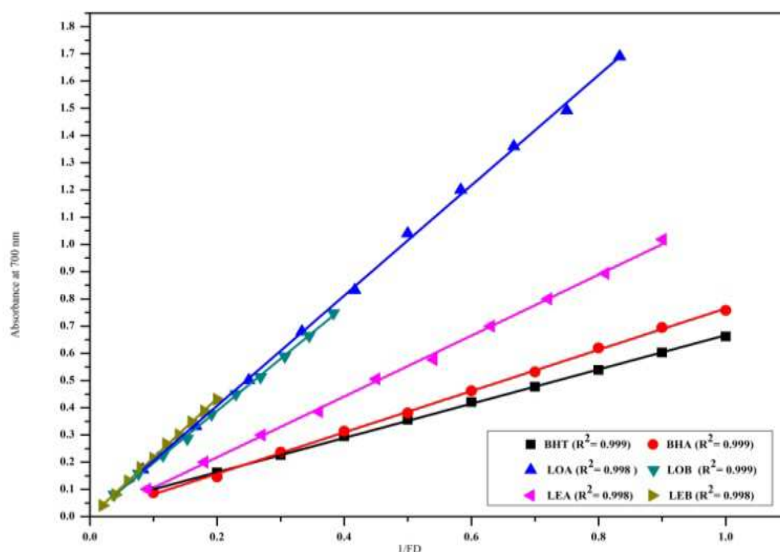

Figure 4: Comparison of DPPH radical scavenging activity of fractions L.guyonianum

3.5.2. Reducing power assay

Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action [25].In the reducing power assay, the presence of antioxidants in the samples would result in the reducing of $Fe^{3+}$to $Fe^{2+}$by donating an electron. Amount of Fe^{2+}complex can be then be monitored by measuring the formation of Perl's Prussian blue at 700nm [26].

reducing power ranging from (0.55 ± 0.04 to 2.16 ± 0.13 mM) showed higher reducing power in *LE* butanol fraction(2.16 ± 0.13 mM) and lower in *LE* ethyl acetate fraction(0.55 ± 0.04mM)(Table 3).The reducing powers for the different extracts were in the following order: *LO* >*LE* for ethyl acetate fraction. Whilst *LE* >*LO* for butanol fraction.

Figure5 shows the absorbance changes within the concentration of the extracts expressed as the inverse of the dilution factor (1/FD) of the extracts. The results of this research showed that the reducing power the extracts are all surpassed in its ability to the reducing power compared with each of the BHA and BHT.

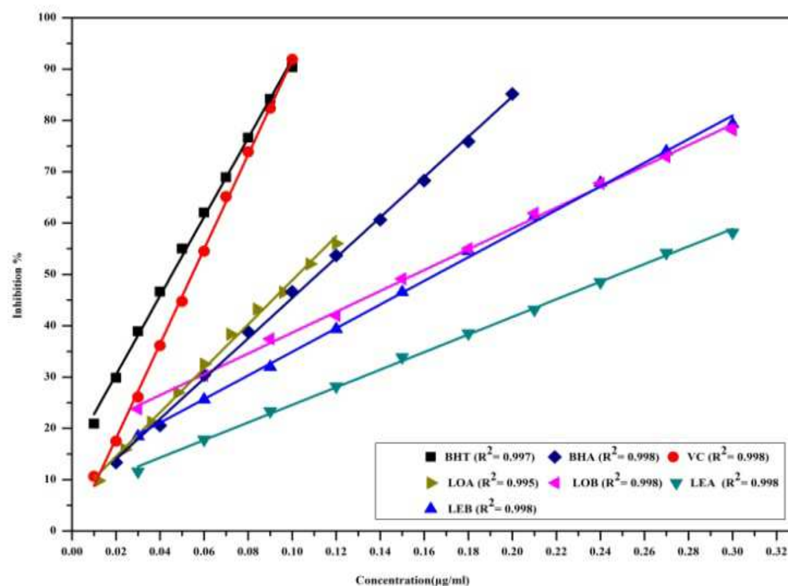

Figure 5: Reducing power of *L. guyonianum*

3.5.3. Phosphomolybdenum Assay (PM)

Total antioxidant capacity by Phosphomolybdenum method assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/Mo (V) complex at acidic pH. The Phosphomolybdenum method is quantitative since the total antioxidant activity is expressed as the number of equivalents of ascorbic acid [27]

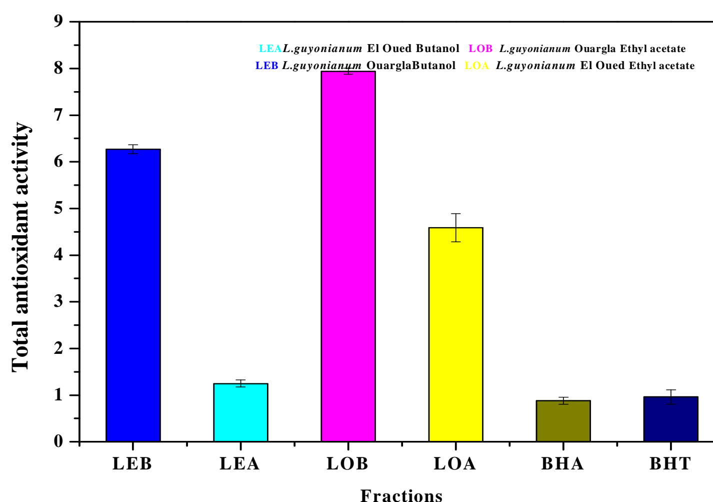

Figure 6: Total antioxidant activity of *L. guyonianum*.

As shown in Table 3 the antioxidant activity of the plant extracts of *L. guyonianum* ranged from (1.25 ± 0.07to 4.59 ± 0.3mM) of ethyl acetate fraction (6.27 ± 0.1 to 7.94 ± 0.06mM).

The *LO* butanol fraction showed the highest while the *LE* ethyl acetate fraction showed the lowest antioxidant potential (Figure 6).

CONCLUSION

Phytochemical analysis results revealed that certain parts of the plant gave a positive test for a particular class of secondary metabolites whereas other parts gave negative test. *LE* butanol fraction contained higher amounts of total phenolics while flavonoids content was higher in *LO* butanol fraction extract. The results of the study show that *L. guyonianum* possesses significant free radical scavenging properties a higher antioxidant activity was found *LO* ethyl acetate fraction for all assays, except for the Phosphomolybdenum method, in which the *LO* butanol fraction showed the best results.

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