Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(6):887-893

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Effects of altitude on the chemical composition and on some biological properties of Lebanese *Eryngium creticum* L.

Hassan Rammal[1,2*], Hussein Farhan[1], Nadia Jamaleddine[1], Majid El Mestrah[1], Mohamad Nasser[1,2] and Akram Hijazi[1]

[1]*Doctoral School of Science and Technology, Research Platform for Environmental Science (PRASE), Faculty of Sciences, Lebanese University, Beyrouth, Lebanon*
[2]*Faculty of Agriculture and Veterinary Science, Department of Basic Sciences, Lebanese University, Beyrouth, Lebanon*

ABSTRACT

The present work aimed to evaluate the antioxidant and antiproliferative properties of leaves and stems of the Lebanese Eryngium creticum grown in Bekaa at 1200 m and then to compare the obtained results with those obtained using the same plant grown up at 300 m in order to determine the effects of the altitude on the chemical composition and thus on the biological properties of this plant. Phytochemical screening results show that the two studied parts of E. creticum are rich in a variety of secondary metabolites depending on the used solvent. In addition, an antioxidant activity arises due to phenolic compounds present. Increasing number of RAW264.7 cells showed some anti-inflammatory properties. At the same time, an antiproliferative capacity was observed using MCF-7 and MDA-MB-468 cells. All of these results have been compared with others obtained using the same plant growing at 300 m. Our study showed that the altitude exerts an effect on the chemical composition of the plant and thus on its biological activities.

Keywords: *Eryngium creticum*, phytochemical screening, Antioxidant activity, anti-inflammatory property, anti proliferative property, Altitude.

INTRODUCTION

During the last decade there had been many temptations to incorporate medicinal plants in modern medical systems and the reasons are many as their lower cost and their availability regarding the cost of the pharmaceutical products which is still rising for governments and individuals. On the other hand, the existence of diseases for which there is no effective pharmaceutical treatments has opened hope in these plants for healing, and there was a lot of laboratory confirmations on the efficacy of an increasingly number of medicinal plants [1].

Despite that Lebanon is considered small, it is rich in many plants with medicinal properties. 2607 wild species from which 92 are endemic, hence the importance of using these plants and expand the investigation of their biological properties.

Eryngium creticum is perennial plant that belongs to Umbellifereae family. It is found only in Lebanon, Palestine, Jordan and Syria. It is cultivated for use as vegetable mainly in salad. It is traditionally used as diuretic, laxative. Submerged roots and seeds in water have been drunk to treat the kidney stone and the infections, skin diseases and tumors. It is an antidote, used in the treatment of the snakebite [2]. *E. creticum* showed also an anti-inflammatory property and an anti-microbial activity [3]. It was also used in the treatment of liver diseases, poisoning, anemia and infertility [4]. This plant has showed an antioxidant property by inhibiting the lipid peroxidase in the liver of the rat

[5]. Recently, a study demonstrated the antioxidant and anti-tumor activities of the Lebanese *E. creticum* grown at 300 m [6,7].

Our work aims to explore, for the first time, the importance of *E. creticum* grown in Bekaa at an altitude of 1200 meters and to compare the results with those of the same plant studied before [6,7] grown at an altitude of 300 meters in southern Lebanon to assess the effect of the altitude on the chemical composition and so on some biological properties.

EXPERIMETAL SECTION

Plant collection and preparation of powders

Fresh *Eryngium creticum* was gathered from Bekaa, Makneh at 1200 m altitude during April 2014. Then, plants were well cleaned and washed with water and then dried in the shade and at room temperature and dried inside the limit in humid, well-opened to prevent damage happen to samples. After this period, leaves and stems of the collected plant have been grinded and transformed to powder by a grinder. The powders were preserved in clean plastic containers, kept away from light and heat and moisture until use.

Preparation of extracts

100 g of each part of the plant were put in 500 mL of the selected solvent (water, ethanol and methanol). After a period of maceration and stirring for 8 hours at room temperature and then at 37 °C, the macerate obtained was filtered to remove insoluble residues. The filtrate was then condensed with a rotary evaporator to half evaporation and the filtrates were then frozen before being lyophilized powder to be processed.

Phytochemical Screening

To study the chemical composition (Alkaloids, Tannins, Resins, Saponins, Flavonoids, Terpenoids, Volatile oils, Carbohydrates and Phenols) of the aqueous, ethanolic and methanolic extracts of the leaves and stems of *E. creticum*, qualitative detection of secondary metabolites was performed according to Muanda [8].

Total phenolic compounds (TPC)

The Folin–Ciocalteau reagent method has been used for the estimation of TPC [9]. Five concentrations of all extracts of the used plants have been prepared and then 100 µL have been taken from each concentration and mixed with 0.5 mL of Folin–Ciocalteau reagent (1/10 dilution) and 1.5 mL of Na_2CO_3 2% (w/v). The blend was incubated in the dark at room temperature for 15 min. The absorbance of blue-colored solution of all samples was measured at 765 nm using a Gene Quant 1300 UV-Vis spectrophotometer. The results were expressed in mg of Gallic acid equivalent (GAE) per g of dry weight of plant powders.

Total flavonoids compounds (TFC)

The aluminum chloride method [10] was used for the determination of TFC of all extracts of the studied plant. 1 mL of various concentrations of all extracts was mixed with 1 mL of 2 % methanolic aluminum chloride solution. After an incubation period at room temperature in the dark for 15 min, the absorbance of all samples was determined at 430 nm using a Gene Quant 1300 UV-Vis spectrophotometer. The results were expressed in mg per g of rutin equivalent (RE) and ethanol was used as blank.

Evaluation of the antioxidant activity

The method of Farhan et al. [9] has been used for the scavenging ability of DPPH antioxidant test. 1 mL of different concentrations of diluted extracts of the plant parts in used solvent was added to 1 mL of DPPH (0.15 mM in same solvent) and at the same time, a control consisting of 1 mL DPPH with 1mL of used solvent was prepared. The reaction mixtures were mixed very well by hand and then incubated in the dark at room temperature for 30 min and the absorbance was measured at 517 nm by a Gene Quant 1300 UV-Vis spectrophotometer. The DPPH scavenging ability of plant extracts was calculated using the following equation:

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

The A control is the absorbance of DPPH + used solvent; A sample is the absorbance of test sample.

Evaluation of the anti-inflammatory activity

To investigate the anti-inflammatory activity a cell culture was conducted using RAW264.7 macrophage cells. Then the measure of viability and cell proliferation was made by the XTT technique. Briefly, cell culture was performed in 96-well plates each containing 100 µL DMEM equivalent to 20.000 cells. The extracts were diluted with the DMEM culture medium at decreasing concentrations (200, 100, 50, 25 and 5 µg/mL) and are then added to the wells

after pre-incubation of the cells for 24 hours. The plates were then incubated under 5% CO_2 and at a temperature of 37 °C for 24 and 48 hours. Then 50 µL of XTT solution has been added to each well followed by an incubation for 24 and 48 hours. Finally, the absorbance was read at 490 nm.

Evaluation of the antiproliferative activity

To study the antiproliferative activity of leaves and stems of *E. creticum* a cell culture was performed using breast cancer cell MCF-7 and MDA-MB-468 cells of the mammary gland / breast; derived from the metastatic site: pleural effusion. Then a measure of the inhibition of cell proliferation by the neutral red method was applied.

Cell culture was performed in 96-well plates each containing 100 µL DMEM equivalent to 20.000 cells. The extracts were diluted with the DMEM culture medium at decreasing concentrations (200, 100, 50, 25 and 5 µg/mL) and are then added to the wells after pre-incubation of the cells for 24 hours. The plates were then incubated under 5% CO_2 and at a temperature of 37 °C for 24 and 48 hours. Then Neutral Red (NR) solution has been added to each well followed by an incubation for 3 hours. After the time of contact with xenobiotics, the amount of NR incorporated into the cells was measured by an ELISA reader with a spectrophotometer at 540 nm. This quantity is directly proportional to the number of cells with an intact membrane [6].

Statistical analyses

Data are presented as mean ± standard deviation (SD) of three independent experiments and statistical significance was determined using the unpaired t-Test using the software Prism 6.0 (GraghPad Software, Inc.). A significant difference was considered if $P < 0.05$.

RESULTS AND DISCUSSION

Phytochemical screening

In order to study the chemical composition of various extracts prepared from stems and leaves of the plant *E. creticum* grown at an altitude of 1200 m and compare it with the same plant that grows up at an altitude of 300 m, a standard phytochemical screening has been practiced.

Table 1: Phytochemical screening of *E. creticum* leaves and stems aqueous extracts

	Altitude			
	1200 m		300 m [6]	
	Leaves	Stems	Leaves	Stems
Alkaloids	-	-	+	+
Tannins	+	-	++	+
Resins	+	+	-	-
Saponins	-	-	+++	+++
Phenols	++	+	++	+
Terpenoids	+	+	++	++
Flavonoids	+	++	-	-
Carbohydrates	-	-	-	-
Volatile oils	-	-	-	-

(+++) sign indicates a very positive response; (++) sign indicates a positive response; (+) sign indicates a moderately positive response; (-) sign indicates a negative response so the absence of chemical constituents

Table 2: Phytochemical screening of *E. creticum* leaves and stems ethanolic extracts

	Altitude			
	1200 m		300 m [6]	
	Leaves	Stems	Leaves	Stems
Alkaloids	-	+	++	+
Tannins	+	-	+	-
Resins	+	-	++	+
Saponins	-	-	++	++
Phenols	++	++	+++	+
Terpenoids	+++	++	++	+
Flavonoids	-	+	++	++
Carbohydrates	+	++	-	++
Volatile oils	-	-	-	-

(+++) sign indicates a very positive response; (++) sign indicates a positive response; (+) sign indicates a moderately positive response; (-) sign indicates a negative response so the absence of chemical constituents

Table 3: Phytochemical screening of *E. creticum* leaves and stems methanolic extracts

	Altitude			
	1200 m		**300 m** [6]	
	Leaves	**Stems**	**Leaves**	**Stems**
Alkaloids	-	+	++	++
Tannins	+	-	+	+
Resins	-	-	++	+
Saponins	-	-	-	-
Phenols	++	++	+++	++
Terpenoids	+++	++	+++	++
Flavonoids	++	+	++	++
Carbohydrates	+	+	/	/
Volatile oils	-	-	-	-

(+++) sign indicates a very positive response; (++) sign indicates a positive response; (+) sign indicates a moderately positive response; (-) sign indicates a negative response so the absence of chemical constituents

The results obtained from the phytochemical screening presented in Tables 1, 2 and 3 show that *E. creticum* is rich in a variety of secondary metabolites depending on the studied part (leaf or stem) and on the used solvent. We noted the presence of resins, terpenoids, flavonoids and phenol in the aqueous extracts of the plant grown at 1200 m, whereas the majority of other secondary metabolites appear more significantly in the ethanolic and methanolic extracts. Thus there is a difference of the presence and amount of secondary metabolites in the same plant between the stems and leaves using the same solvent in favor of the leaves that are richer in secondary metabolites than stems which gives them a greater bioavailability. Consequently, with its wealth in secondary metabolites, *E. creticum* may present several medical importances. It can be regarded as a analgesic due to the presence of alkaloids [11]. In addition, the presence of flavonoids in the plant gives it an antitumor property [12] and an antioxidant property. On the other hand, we noticed a difference in the amount of secondary metabolites between *E. creticum* that grows at 1200 m and that grows at an altitude of 300 m in favor of that of 300 m. This difference may be due to the acclimations of the plant to a decreased temperature, low oxygen and low atmospheric pressure that result in a change in the amount of secondary metabolites. Also, this difference may be explained by the fact that at 1200 m we can observe a high amount of snow however at 300 m we note the presence of high quantity of rain, factors that can change the chemical composition of the different parts of plants.

Determination of total phenolic contents (TPC) and total flavonoids contents (TFC)

The TPC and TFC in the leaves and stems of *E. creticum* were evaluated. The concentrations of the different extracts for leaves and stems were represented in Table 4. The absorbance of these extracts, with an absorbance of 765 nm for the TPC and 430 nm for the TFC was measured in triplicates experiments for two different concentrations.

As shown in Table 4, both aqueous, MeOH and EtOH extracts from both leaves and stems of this plant were found to contain high amounts of TPC and TFC in the EtOH extract than the two other extracts. Moreover, the amounts of TPC and TFC in the leaves are higher than the stems.

In comparison with the TPC and TFC evaluated for the plant grown at 300m we can find that this later has higher amount that *E. creticum* grown at 1200 m [7].

Table 4: TPC and TFC of different solvent extracts from leaves and stems of *E. creticum*

	Aqueous Leaves	**Aqueous Stems**	**Ethanolic Leaves**	**Ethanolic Stems**	**Methanolic Leaves**	**Methanolic Stems**
TFC	0.93 %	4.41 %	17.53 %	3.75 %	16.80 %	3.55 %
TPC	33.88 %	31.96 %	24.19 %	21.85 %	17.27 %	14.33 %

Antioxidant activity

Our results presented in table 5 showed an increase in the antioxidant activity of the leaves and stems of *E. creticum* with increasing the concentration of the aqueous extract. This increase has reached 59 % and 35 % at the concentration 0.5 mg/mL for the leaves and stems respectively. These results may be due to the fact that the leaves contain more polyphenols that stems as shown in table 1.

Studies on the same plant but from an altitude 300 m showed that the leaves and stems at the same concentration (0.5 mg/mL), exerted an antioxidant activity of 90 % and 75 % respectively. These results show that the altitude has a significant influence on the chemical composition of the plant. It has induced a decrease of flavonoids and polyphenols which have an important role in the antioxidant power and therefore it has caused a reduction in the antioxidant capacity [6].

On the other hand, at a concentration of 0.5 mg/mL the ethanolic extract showed an important antioxidant activity of both leaves (81 %) and stems (79 %) (Table 5). However, this antioxidant capacity was highest for the plant that grows at 300 m. It has reached the 93 % for the leaves and 82 % for the stems [6].

For the methanolic extract, the obtained results showed that both leaves and stems have exerted an important antioxidant activity reaching 90 % and 79 % respectively at a concentration of 0.5 mg/mL (Table 5). These results may be due to the presence of higher amount of flavonoids and phenols and both parts.

Table 5: Free radical scavenging activity of *E. creticum* leaves and stems in DPPH assay

	% of Scavenging activity					
Concentration (mg/mL)	**Aqueous Leaves**	**Aqueous Stems**	**Ethanolic Leaves**	**Ethanolic Stems**	**Methanolic Leaves**	**Methanolic Stems**
Control	-	-	-	-	-	-
0.1	20	7	53	21	80	29
0.2	33	14	60	42	85	39
0.3	48	22	78	63	86	58
0.4	53	26	78	77	88	62
0.5	59	35	81	79	90	79

% Scavenging activity = [(A control (517)−A sample (517)) / A control (517)] ×100

Anti-inflammatory Activity

For the first time, an evaluation of the anti-inflammatory activity of each of the extracts prepared using different solvents from leaves and stems of *E. creticum* has been done by measuring the viability of the RAW264.7 cell line after 24 hours treatment with increasing concentrations (5, 25, 50, 100, and 200 µg/mL) of these extracts.

The cell viability XTT test shows that aqueous extracts of the leaves and stems of *E. creticum* increased the number of macrophages (RAW 264.7) with increasing concentrations of the extract from 5 to 200 µg/mL compared to control as shown in table 6.

Table 6: Effects of aqueous, ethanolic and methanolic extracts from leaves and stems of *E. creticum* on RAW264.7 cell line after 24 hours treatment (*P<0.05*)

	Number of cells					
Concentration (µg/mL)	**Aqueous Leaves**	**Aqueous Stems**	**EtOH Leaves**	**EtOH Stems**	**MeOH Leaves**	**MeOH Stems**
Control	20000	20000	20000	20000	20000	20000
5	23767*	28738**	29366***	25488*	25807**	24287*
25	21928,4	27570**	29565***	28760**	12595	24364*
50	24926**	26501*	26501**	28000**	27945**	24970*
100	28617***	29036***	23537*	19174	29025***	26446,3**
200	26534**	32573***	28265**	15119	17388	28430**

Ethanolic extract of both leaves and stems of the plant has induced an increase in the viability of RAW 264.7 cells, but we observed that the most important viability level is at a low concentrations of the extract (5 and 25 µg/mL) especially from the leaves where the number of cells reached 29366 at a concentration of 5 µg/mL (Table 6).

For the methanolic extract cell viability XTT test shows that stems of this plant have increased the number of cells till 28430 at the concentration of 200 µg/mL. On the other hand, methanolic extract from leaves of this plant has increased the number of cells at the concentrations of 5, 50 and 100 µg/mL but it decreased this number at the concentrations of 25 and 200 µg/mL as shown in table 6.

Antiproliferative activity

An evaluation of the anticancer activity of each of the extracts prepared from leaves and stems of *E. creticum* grown at 1200 m using different solvents was made for the first time by measurement of cell viability in MCF-7 cell line and MDA-MB-468 cells after treatment for 24 and 48 hours with increasing concentrations (5, 25, 50, 100, and 200 µg/mL) of these extracts.

After 24 hours treatment with the aqueous extract from leaves and stems of *E. creticum* with increasing concentrations, we observed a partial inhibition of the proliferation of cancer cells MCF-7 and MDA. The obtained results showed that leaves of this plant have induced a maximal partial inhibition of the cell line MCF-7 at a concentration of 200 µg/mL where the number of cells was lower than the control. For the MDA cell line we found that the maximum inhibition of the proliferation has occurred at low concentrations (50 and 100 µg/mL) and especially at the concentration of 100 µg/mL as shown in tables 7 and 8. On the other hand aqueous extracts from stems of E. creticum did not exert an antiproliferative effect at all used concentrations.

For the ethanolic extract, after 24 h treatment, it was noted that both leaves and stems of the plant did not exert an antiproliferative effect of the MCF-7 cell line at all concentrations as shown in table 7. On the other hand, leaves at the concentration of 200 µg/mL have decreased the number of MDA cells by 6 % (table 8). Also, stems of this plant have showed a partial inhibition of the proliferation at the concentrations 25 and 100 µg/mL by 18 % and 6 % respectively (Table 8) .

Concerning the methanolic extract, leaves at the concentration 200 µg/mL have induced a significant decrease of the number of MCF-7 and MDA cells by 62 % and 48 % respectively (Tables 7, 8). Also, all concentrations of stems except 25 µg/mL have induced an antiproliferative activity by decreasing the number of MCF-7 cells. The most important concentration was 5 µg/mL which decreased the number of MCF-7 by 28 % as shown in table 7. For the MDA cells, methanolic extract of stems has only decreased the number of cells at the concentration 5 µg/mL. This decrease reached 33 % as shown in table 8.

Table 7: Antiproliferative activity of aqueous, ethanolic and methanolic extracts from leaves and stems of *E. creticum* on MCF-7 **cell line after 24 hours treatment**

	Number of cells					
Concentration (µg/mL)	**Aqueous Leaves**	**Aqueous Stems**	**EtOH Leaves**	**EtOH Stems**	**MeOH Leaves**	**MeOH Stems**
Control	20000	20000	20000	20000	20000	20000
5	34025	30629	32075	30063	20881	14402*
25	21006	38239	30187	25978	23648	27170
50	22075	30566	32201	25975	29497	16918
100	19308	32704	29245	26918	19624	17296
200	14528	29182	31635	27736	7610***	18994

Table 8: Antiproliferative activity of aqueous, ethanolic and methanolic extracts from leaves and stems of *E. creticum* on MDA **cell line after 24 hours treatment**

	Number of cells					
Concentration (µg/mL)	**Aqueous Leaves**	**Aqueous Stems**	**EtOH Leaves**	**EtOH Stems**	**MeOH Leaves**	**MeOH Stems**
Control	20000	20000	20000	20000	20000	20000
5	26097	25072	25869	21880	28775	13333*
25	24729	21823	24106	16467	22849	27464
50	14017*	25413	22792	17493	21595	23248
100	12080*	28433	25242	18747	19373	30199
200	18803	22450	18747	21254	10427**	34017

After 48 hours treatment with the three extracts from leaves and stems of *E. creticum* grown at 1200 m the obtained results by the neutral red technique have showed that the number of treated cells is lower than the control for both MCF-7 and MDA cell lines as shown in tables 9 and 10. The most important decrease was noted for the concentration 200 µg/mL of both leaves and stems for all extracts.

Table 9: Antiproliferative activity of aqueous, ethanolic and methanolic extracts from leaves and stems of *E. creticum* on MCF-7 **cell line after 48 hours treatment**

	Number of cells					
Concentration (µg/mL)	**Aqueous Leaves**	**Aqueous Stems**	**EtOH Leaves**	**EtOH Stems**	**MeOH Leaves**	**MeOH Stems**
Control	33000	33000	33000	33000	33000	33000
5	21119	16973*	20531*	24089	22000	29638
25	23991	23861	19944*	20498	24024	20074*
50	18279*	18769*	19552*	22816	24970	24546
100	18834*	17822*	25362	14460**	20662*	22751
200	15537**	12436**	16092**	20401*	13285**	17039*

Table 10: Antiproliferative activity of aqueous, ethanolic and methanolic extracts from leaves and stems of *E. creticum* on MDA **cell line after 48 hours treatment**

	Number of cells					
Concentration (µg/mL)	**Aqueous Leaves**	**Aqueous Stems**	**EtOH Leaves**	**EtOH Stems**	**MeOH Leaves**	**MeOH Stems**
Control	33000	33000	33000	33000	33000	33000
5	17405**	18092*	19901*	23447	17694*	11832**
25	20806*	20842*	19033*	15632**	20372*	19684*
50	15921**	15414**	22109	16862*	17079*	14763**
100	14799**	17730*	17586*	18128*	10313***	13171**
200	16500*	19141*	6007***	15451**	5391***	10204***

CONCLUSION

The antioxidant, anti-inflammatory and antiproliferative activities of aqueous, ethanolic and methanolic extracts from leaves and stems of *E. creticum* grown at 1200 meters was evaluated in this work.

By comparing some of the obtained results with those obtained in another study on the same plant grown at 300 meters, we can estimate that the altitude affects the chemical composition of *E. creticum* and consequently it will influence its biological activities. In conclusion, from a low level to a high altitude the plant loses a certain amount of ingredients and therefore decrease in its medical values.

REFERENCES

[1] PA Cox; MJ Balik, *Sci. Am.*, **1994**, 270, 60-65.
[2] A Abu-Rabia, *Asia Pac. J. Cancer Prev.*, **2005**, 6, 101-107.
[3] MS Ali-Shtayeh; MR Yaghmour; YR Faidi; K Salem; MA Al-Nuri, *J. Ethnopharmacol.*, **1998,** 6, 265-271.
[4] O Said; K Khalil; S Fulder; H Azaizeh, *J. Ethnopharmacol*., **2002**, 83, 251-265.
[5] P Ljubuncic; H Azaizeh; I Portnaya; U Cogan; O Said; K Saleh et al, *J. Ethnopharmacol.*, **2005**, 99, 43-47.
[6] Z Dirani; R Makki; H Rammal; S Naserddine; A Hijazi; HF Kazan; M Nasser; A Daher; B Badran, *Int. J. Biol. Pharm. Allied Sci.*, **2014**, 3(10), 2199-2222.
[7] M Dammous; H Farhan; H Rammal; A Hijazi; A Bassal; HF Kazan; Y Makhour; B Badran, *Int. J. Sci.*, **2014**, 3(4), 40-53.
[8] F Muanda. Identification of polyphenols, evaluation of their antioxidant activity and study of their biological properties. Thesis, University of Metz, France, **2010**, 1-239.
[9] H Farhan; H Rammal; A Hijazi; H Hamad; B Badran, *Ann. Biol. Res.*, **2012**, 3(1), 149-156.
[10] C Quettier-Deleu; B Gressier; J Vasseur; T Dine; C Brunet; M Luyckx; M Cazin; JC Cazin; F Baileul; F Trotin, *J. Ethnopharmacol*., **2000**, 72, 35-42.
[11] M Khader; N Bresgen; PM Eckl, *J. Ethnopharmacol*., **2010**, 127, 319-324.
[12] C Kandaswami; LT Lee; PP Lee; JJ Hwang; FC Ke; YT Huang; MT Lee, *In Vivo*, **2005**, 19(5), 895-909.