Journal of Multidisciplinary Engineering Science and Technology (JMEST)
ISSN: 2458-9403
Vol. 3 Issue 11, November - 2016

Antioxidant Activity And Chemical Composition Of The Ethanolic Extract From Leaves And Stems Of The Lebanese *Eryngium Creticum*

Raghida Damaj[1], Abbas Sabbah[1,2], Ghassan Nasser[1,2], Mira Bou Francis[1], Akram Hijazi*[1], Hussein Annan[1], Abd-Al-Ameer Al Rekaby[3], Hassan Rammal*[1,2]

1- Plateforme de recherche et d'analyse en sciences de l'environnement (EDST-PRASE), Beirut, Lebanon.

2- Faculty of Agriculture, Department of fundamental sciences, Lebanese University

3- Al Mustansiriya University, College of Science, Department of Biology, Irak.

*Corresponding author. E-mail: hijazi_akram@hotmail.com, Akram Hijazi; Phone: +961 71905768

hasanrammal@hotmail.com

***Abstract*—First to discuss different extraction techniques along with their basic mechanism for extracting bioactive compounds from leaves and stems of the Lebanese Eryngium creticum L. Second, to purify, identify and quantify some of the bioactive compounds from the ethanolic extracts of leaves and stems of this plant and to evaluate its sterols and fatty acids content. Finally to estimate its antioxidant activity. Purification, identification and quantification of the bioactive compounds of leaves and stems of the studied plant have been done using a chromatographic analysis. The antioxidant activity was evaluated using a spectrophotometric method. Finally, the analysis of fatty acids and sterols has been estimated using different techniques such as GC-MS and HPLC-DAD.The phytochemical screening indicates that E. creticum contains high levels of minerals and bioactive compounds. On the other hand, using the DPPH test, the ethanolic extract showed high antioxidant capacity dependent on the concentration. These results show that E. creticum is a good source of different antioxidant and bioactive compounds.Ethanolic extracts from both parts of this plant have a good antioxidant power, so it can be used as a source of multi drug resistant in the future.**

Keywords—Eryngium creticum L.; extraction techniques; sterols; fatty acids; antioxidant activity.

I. INTRODUCTION

Since the beginning of the century, new techniques were used to extract many natural substances having active principles, and to highlight the pharmacological properties of plants, which represent an inexhaustible and renewable source. Natural substances are experiencing growing interest for applications in the production of many consumer products. In fact, their use is encouraged as the equivalent products from chemical syntheses have, rightly or wrongly, bad reputation among the general public.

Eryngium creticum or *Kors Anné* in Arabic is a vivacious plant that belongs to the family Umbelliferae. It exists only in Lebanon, Palestine, Jordan and Syria [1],2]. It is cultivated as a vegetable and it is especially consumed as salad. It is traditionally used as a diuretic and even as a laxative. It is an antidote used in the treatment of snake bites[3]. The roots and grains submerged in water are used to treat kidney stones and infections, skin diseases and tumors. It also showed anti-inflammatory and antimicrobial activities [4]. In addition, it has been used in the treatment of liver diseases, intoxication, anemia and infertility. It also showed antioxidant property by the inhibition of lipid peroxidase in rat liver [5]. Also, recent studies have demonstrated the antioxidant power of different extracts from parts of this plant [6].

Our present work is aimed first to extract some active compounds using different extraction techniques. Secondly, to evaluate the antioxidant activity of the ethanolic extract from both leaves and stems of this plant. And finally, to describe the structural characterization of isolated compounds, realized by using spectroscopic analysis methods: 1D NMR (^{1}H and ^{13}C).

II. MATERIAL and METHODS

A. Plant collection

Fresh plant was gathered from northern Lebanon Batroun, from a region named Yarita at 565 m of altitude. Then, the plant parts were well cleaned and washed with water then dried in the shade at room temperature for two weeks. After this period, the leaves and stems of the plant were crushed up and ground to get homogeneous fine powder by a grinder and then kept in a dark place at room temperature till use in the different studies.

B. Apparatus and chemicals

All the chemicals used were of analytical grade. Ethanol, hexane, methanol, dichloromethane and acetone were purchased from BDH England, silica gel

was purchased from Merck Germany, hydrogen peroxide was purchased from Unichem India, DPPH was purchased from Sigma Aldrich, USA, ^{1}H NMR and ^{13}C NMR spectra were recorded on an Avance III (300) spectrometer at 300 MHz.

C. Types of extraction

C.1. Maceration process

A total of 5 g of each powdered leaves and stems of *E. creticum* were put into a flask with 250 mL of ethanol, and the mixture has been extracted by agitation for 48 h under continuous agitation (360 rpm) at room temperature in the dark. Subsequently, the extracts were collected and filtered on a Buchner. Then the extracts were concentrated using a rotary evaporator at 40 °C under reduced pressure. Finally, the extracts were weighed and stored at -20 °C till their usage in different tests [8].

C.2. Reflux Method

250 mL of ethanol were added to 5 g of powdered leaves and stems of *E. creticum* into a round bottomed flask. The mixture was refluxed for 4 h. Thereafter, the extracts were filtered using a Buchner funnel under vacuum and concentrated using a rotary evaporator at 40 ^{0} C under reduced pressure. Once completed, the residue was weighed and stored at -20 °C till their usage in different tests[8].

C.3. Soxhlet process

5 g of powdered leaves and stems were extracted in 250 mL of ethanol by the soxhlet extraction technique for 4 h. The extracts were filtered and the filtrate was evaporated under reduced pressure and then stored at -20 °C[8].

C.4. Ultrasound assisted extraction (UAE)

5 g of powdered leaves and stems in 250 mL of ethanol was put for 1 hour at 60 ^{0} C in the ultrasound apparatus, then filtered and evaporated to be stored -20 °C[8].

D. Fatty acid analysis by GC-MS

D.1. Fatty acid analysis

The fatty acid content was determined by analysis of methyl esters of fatty acids by gas chromatography (GC) according to standard NF EN ISO 5508 which requires two preliminary steps: the extraction and the esterification of fatty acids. The extraction of fatty acids corresponds to the solubilization of triglycerides contained in the oil in an organic solvent. This step is followed by methyl-transesterification which consists in adding a methyl group to the released fatty acids to give fatty acid methyl esters to facilitate the separation. Indeed, this derivatization allows making the fatty acids more volatile, ensuring the stability required for GC analysis[9].

20 mg of seed oil extracted by soxhlet are weighed in a test tube. The oil was dissolved in 5 mL of petroleum ether. After agitation, 100 µL were removed from the mixture and transferred to a vial suitable for GC analysis.

D.2. Analysis conditions of profile of fatty acids

150 µL of oil / petroleum ether from the extraction of fatty acids on oil are trans-esterified by addition of 50 µL of trimethyl ammonium hydroxide (TMAH) in methanol. This reaction took place at the time of injection under the effect of temperature and it forms methyl esters of fatty acids. The profile of oil is carried out by GC under the following conditions:

• Column: 30 × 0.25 mm ID. 0.25 microns SLB-5 MS (Sigma -Aldrish)

• Carrier gas: Helium. Column head pressure of 100 kPa.

• Oven temperature: Temperature gradient 50 °C / minute for one minute and then raising the temperature from 50 to 200 ° C at 25 °C / min and then up 230 ^{0} C / min and finally maintained at 230° C for 18 minutes.

• Injector: Splitless 250 ^{0} C, injected volume: 1 µL,

• Detector: MS, temperature of the ionization source: 280^{0} C, electronic ionization (EI) mode.

The data is processed using the Star software. The fatty acids were identified by comparison of retention times with those of a mixture of reference fatty acids.

D.3.Determination of the phytosterol content by GC-MS

100 mg of seed oil were saponified by addition of 2 mL of KOH (1 M) in ethanol, followed by shaking on a vortex and heating for 20 minutes at 75 °C. After cooling, the successive addition of 1 ml of distilled water and 6 ml of cyclohexane, followed by decantation, allows to obtain two phases on which the upper hexane phase was removed. The addition of cyclohexane, the decantation and the removal of the upper phase were repeated 2 times. The two upper phases were combined and 160 µL were taken in a microtube for analysis by GC [10].

D.3.1. Sterols analysis

10 mg of the extract were separated by chromatography on a phase silica gel column and normal pressure. Elution was carried out successively, with solvents of increasing polarity: Petroleum ether, petroleum ether / ethyl acetate, ethyl acetate / methanol, and finally pure methanol. 50 mL per fractions were collected at each time and examined by thin layer chromatography (TLC). The plates were visualized under UV light (254 nm - 366 nm), and then revealed with anisaldehyde, after collection of fractions exhibiting similarities, giving 12 lots[11].

Lot 1 shows, by TLC, a major compound invisible in UV and giving a violet color after revelation with anisaldehyde. Elution was carried out with petroleum ether / ethyl acetate (95/05). This fractionation followed by another chromatographic purification thick layer (CEC) yielded in a pure state a pure product of mass 30 mg (compound A) [11].

Batch 2 of the same extract (petroleum ether extract: elution is carried out each time by a solvent gradient: petroleum ether / ethyl acetate (85/05) - petroleum ether / ethyl acetate (80/20). After exposure to light (254 nm - 366 nm) and by using the anisaldehyde, the compound B is recovered. This latter obtained has required a purification by chromatography on a thick layer, 20 mg of compound B were obtained. Then, a few mg of compound C were separated. The purified and separated fractions are then subjected to NMR and GC-MS analyzes.

D.3.2. Analyses of sterols by GC

The determination of phytosterols was performed with a method developed by Malcherery-Nagel®. At the time of injection, the phytosterols are silylated by adding 40 µL of silylation reagent (BSTFA 1% TMCS).

• Injected volume: 1µL,

• Settings: same conditions as those of fatty acids.

D.3.3. Phytosterols analysis by HPLC-DAD

0.5 g of powder was weighed into an erlenmeyer flask and saponified with 40 mL of ethanolic KOH (10%) for 1 h with constant agitation (1000 rpm) and at 70 °C. After cooling at room temperature, the mixture was centrifuged (Neofuge 23R, Shanghai Lishen Scientific Equipment Co., Ltd., China) for 6 min at 2000 rpm, then decanted and transferred to a separation funnel for extracting the unsaponifiable fraction with 3 x 10 mL of n-hexane. The organic phase was dried with anhydrous sodium sulfate and evaporated to dryness under reduced pressure at 30 °C. The residue was dissolved in 1 mL of a mixture of methanol: acetonitrile (30:70) and then filtered through a membrane of 0.22 µ (Millipore) [11]. The resulting sample is analyzed by HPLC-DAD. The HPLC-DAD system comprises a liquid phase chromatograph equipped with pump models 302 and 305 (Gilson Medical Electronics, Villiers le Bel, France), operating at 20 ^{0} C temperature, flow F = 0.8 mL/min, a length wave length λ = 205 nm, a loop of 20 µL with an isocratic mode and a diode array detector (170, Agilent, Villiers le Bel, France), controlled by Unipoint software system (Gilson Medical Electronics). The separation was performed using a C18 column, 150 mm × 4.6 mm, 4 µm.

E. Antioxidant Activity

Scavenging test of free radical DPPH [12]: The antioxidant test was performed with the DPPH method. 1 mL of each ethanol solution extracts at different concentrations (from 0.1 to 0.5 mg/mL) were added to 1 mL of the ethanolic solution of DPPH (0.15 mM). In parallel, a control was prepared by mixing 1 mL of ethanol with 1 mL of the DPPH solution. The absorbance reading is made against a blank prepared for each concentration at 517 nm after 30 minutes of incubation in the dark at room temperature. The positive control is represented by a standard solution of an antioxidant; ascorbic acid, whose absorbance has been measured under the same conditions as the sample and for each concentration, the test was repeated 3 times. The results were expressed as percent inhibition (I%).

I% = [(Abs control - Abs test) / Abs control] x 100

EC50's values were determined graphically by linear regression.

The standard solution of ascorbic acid was prepared by dilution of 80 µg/mL with ethanol for concentrations ranging from 2.5 to 10 µg/mL.

III. Results and Discussion

A. Comparison of different extraction techniques

The mass of oil extracted is weighed from each extraction technique, and subsequently compared with other techniques in terms of yield (Figure 1). Yields are thus compared with the same techniques performed on other plants in the same extraction conditions (Figures 1 and 2).

The best performance is obtained by the soxhlet method in comparison with that obtained by reflux, sonication and finally maceration (Figure 1). These are confirmed by comparing the obtained results with those of *Urtica dioica* (Figure 2) and even by those of *E. creticum* collected from another region (Rowiest Al Ballout) (Figure 3).

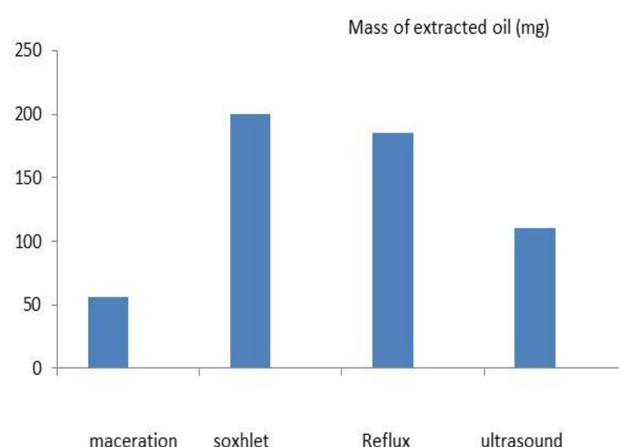

Figure 1. ***Yields of various extraction processes from E. creticum***

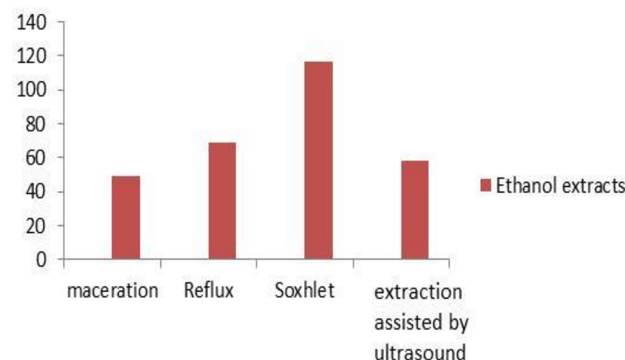

Figure 2. ***The diagram yields extracts from Urtica dioica***

Even compared with extracts of *E. creticum* collected from the Rowiest Al Ballut (Mount Lebanon) [13][14].

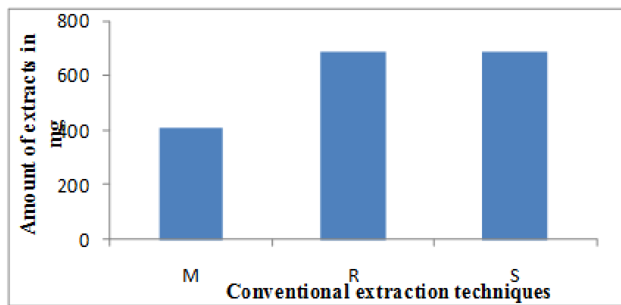

Figure 3. ***Diagram of the extracts yields of E. creticum collected the Rowiest Al Ballut (Mount Lebanon) in February 2014. M= Maceration, R= Reflux and S= Soxhlet***

B. Fatty acid analysis by GC-MS

GC spectra of various samples are presented in Figures 4 and 5. Each retention time (rt) corresponds to one type of fatty acid that the separation of fatty acids is based on their carbon number and number of unsaturation.

- GC of derivatized fraction

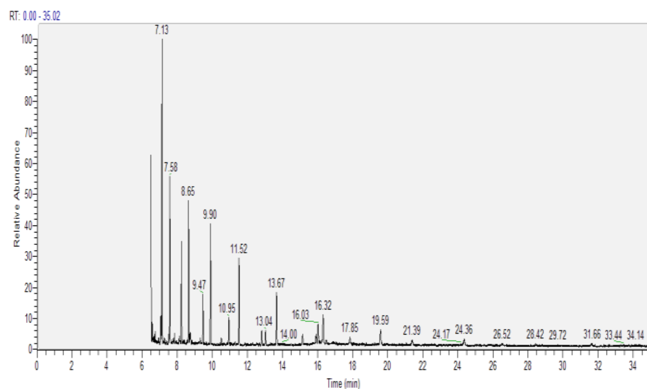

Figure 4. ***GC chromatogram of the derivatized fraction***

- GC of standard methyl esters

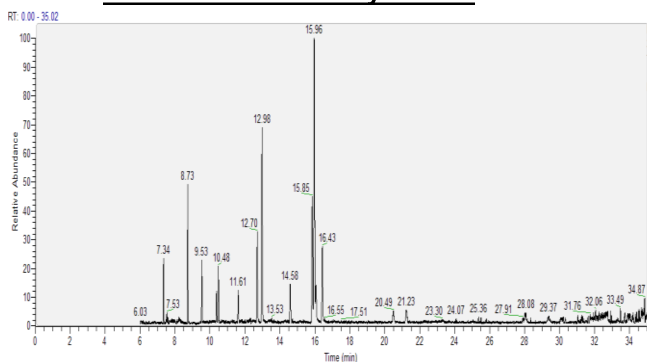

Figure 5. ***GC chromatogram standard methyl esters***

By comparing these two chromatograms it has been characterized the fatty acids in the following amounts:

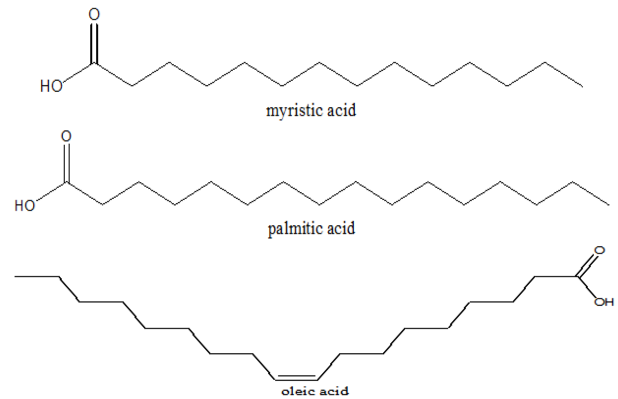

Figure 6. ***Examples of fatty acids obtained by GC-MS***

By integration of the area of each peak to the sum of the peaks, it was possible to quantify the fatty acid content. Each fatty acid is given with its name, the carbon number and degree of unsaturation. Empty boxes indicate the absence of the desired fatty acid. This content is shown in the table below (Table 1).

TABLE 1. ***CONTENT AND FATTY ACID COMPOSITION OF ERYNGIUM CRETICUM***

Fatty acid		Content (%)
C8:0	Caprylic acid	-----
C10:0	Capric acid	-----
C10:1n3	------------	1.022
C10:1n6	------------	-----
C10:2n	------------	-----
C11:0	Heptacosanoic acid	-----
C12:0	Lauric acid	-----
C12:1n6	------------	-----
C13:0	Nonacosanoic acid	-----
C13:1n	------------	1.614
C14:0	Myristic acid	2.383
C14:1n6	------------	-----
C14:1n9	Myristoleic acid	-----
C15:0	Pentadecanoic acid	-----
C15:1	Pentadecenoic acid	3.178
C16:0	Palmitic acid	71.04
C16:1n6	------------	-----
C16:1n9	Palmitoleic acid	-----
C16:2	Hexadecadienoic	2.365
C16:3	------------	-----
C17:0	Margaric acid	0.966
C18:0	Stearic acid	0.548
C18:1n9	Oleic acid	14.90
C18:2n9,12	Linoleic acid	------
C18:3n9,12,15	Linolenic acid	------
C20:0	Arachidic acid	0.122
C20:1	Arachidonic acid	1.022
C20:2	------------	0.996

The fatty acid content is relatively low. Palmitic acid showed the greatest content (71.09%). This is the main product of the synthesis of lipids in our cells. Often symbolized 16: 0 to indicate that it has 16 carbons and no ethylene bond: it is a saturated fatty acid. Its name comes from palm oil, but it is abundant in all animal or vegetable fats and oils. It is an excellent energy food. Industrially, palmitic acid is used for the fabrication of margarines, hard soaps.

C. Sterol analysis by HPLC-DAD

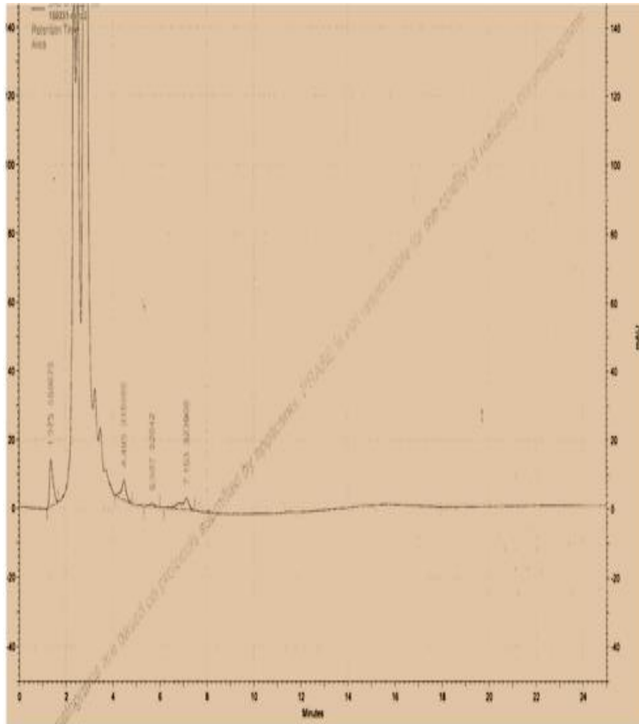

Figure 7. ***HPLC chromatogram of the esterified fraction***

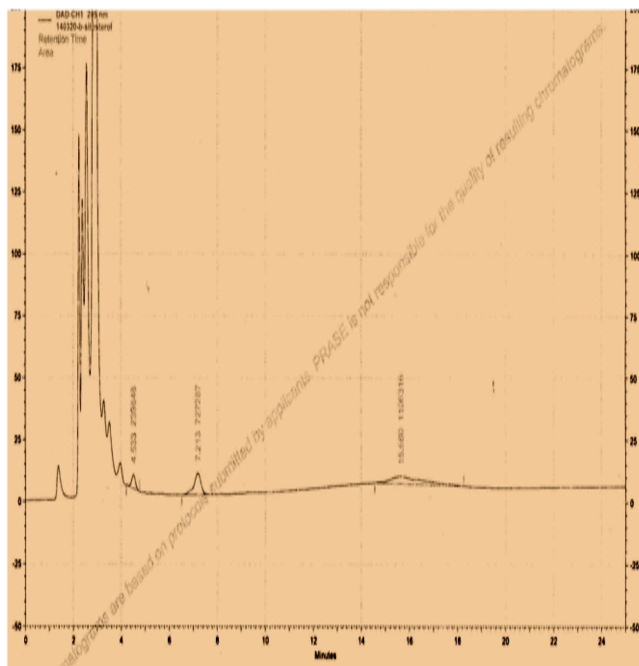

Figure 8. ***HPLC chromatogram of standard stigmasterol***

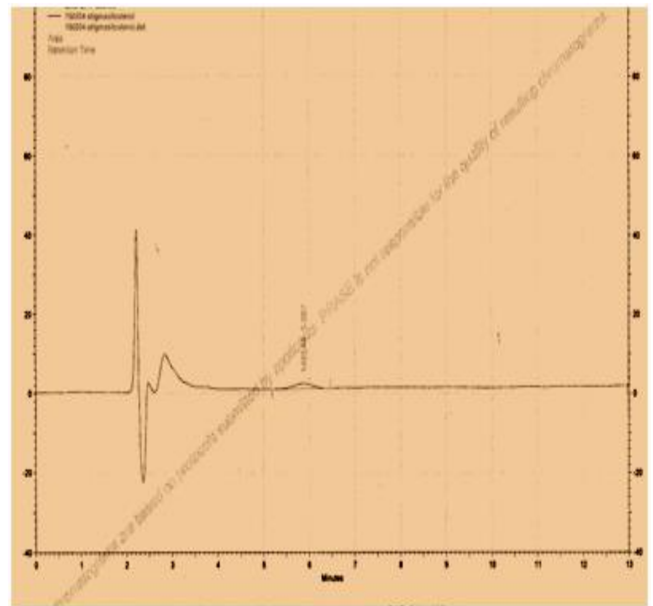

Figure 9. ***HPLC chromatogram of β-sitosterol 75% + standard campesterol 10%***

By comparing the results, the analyzed fraction shows the presence of two types of sterols. The 2 peaks having as rt 4.53 min and 7.21 min (Figure 9), respectively, corresponding to standard β-sitostrol, were found in the derivatized fraction with the same rt approximately 4.49 and 7.15 min (Figure 7), confirming its presence. In addition, the third peak having rt 5.68 min (Figure 7) coincides with the peak correspondent to standard stigmasterol for rt 5.67 min (Figure 8). So the analyzed fraction contains the β-sitosterol and stigmasterol.

D. Analysis and identification of Fractions

<u>* GC of F1</u>

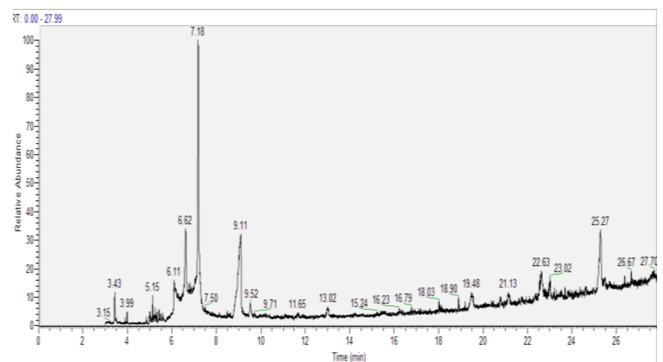

Figure 10. ***GC chromatogram of fraction 1 and its attribution***

As shown in chromatogram: This is the β-sitosterol with a considerable probability. This structure is confirmed by NMR.

- **Result of NMR ^{1}H of the fraction F1:**

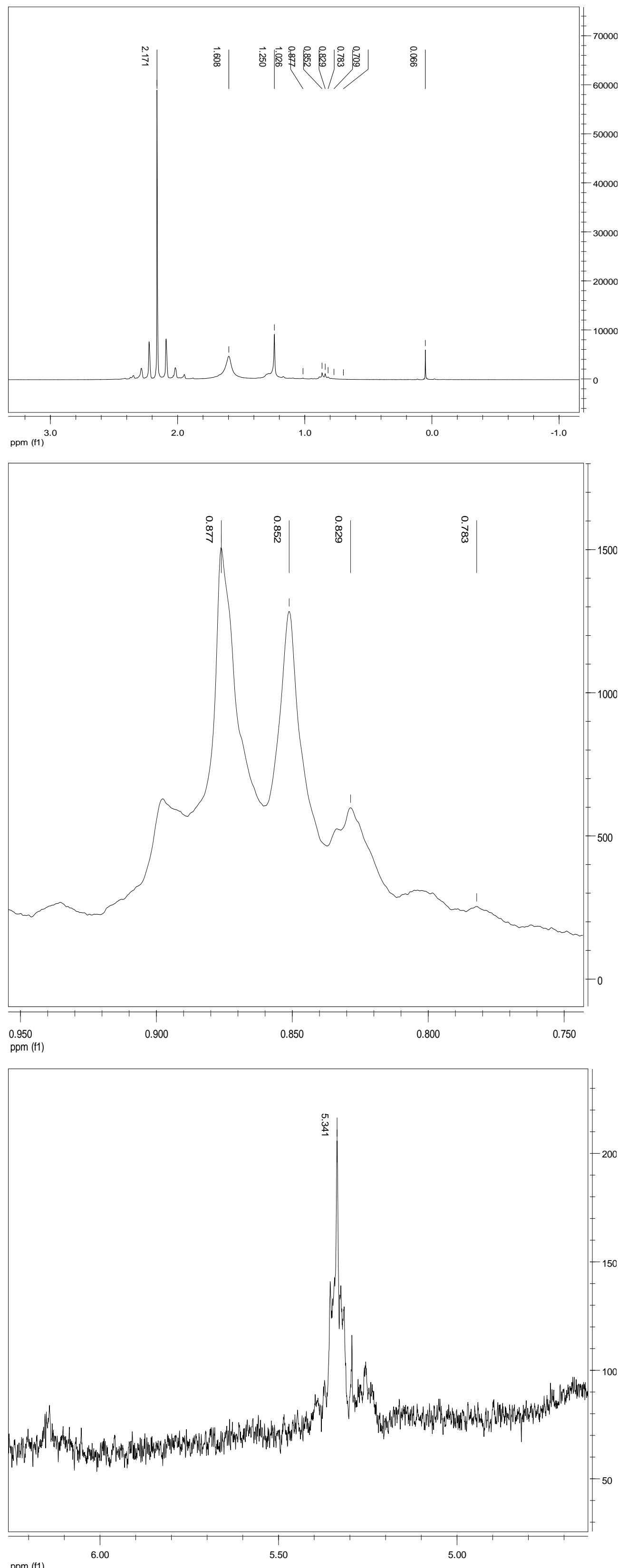

Figure 11. ***Capture of the NMR spectrum of the fraction F1***

Indeed the ^{1}H NMR spectrum recorded at 300 MHz in deuterated chloroform shows:

- Two singlets signals resonate at δH = 0.783 ppm, δH = 1.250 ppm corresponding respectively to two methyl CH_3-18, CH_3-19.

- A multiplet signal at δH= 0.829 ppm, 0.852 ppm, 0.877 ppm assigned to methyl: CH_3-26, CH_3-27, CH_3-29.

- A wide doublet signal at δH = 5.341 ppm characteristic of an olefin proton H-6.

- A singlet signal at δH = 0.783 ppm attributable to methyl CH_3-18.

- A singlet signal resonates at δH = 1.250 ppm corresponding to CH_3-19.

- Finally there is a septet signal (J = 10.5 Hz, J = 5.4 Hz) resonant at δH = 2.171 ppm integrating for one proton, this is the H-3 proton.

- **GC of fraction 2 F2**

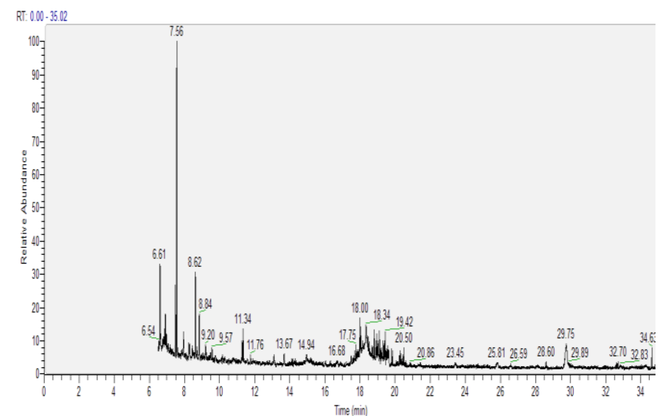

Figure 12. ***GC chromatogram of the fraction F2 and its attribution***

This chromatogram returns to stigmasterol. The latter differs from the β-sitosterol by an olefin bond in position C22.

- **GC of the fraction 3 F3:**

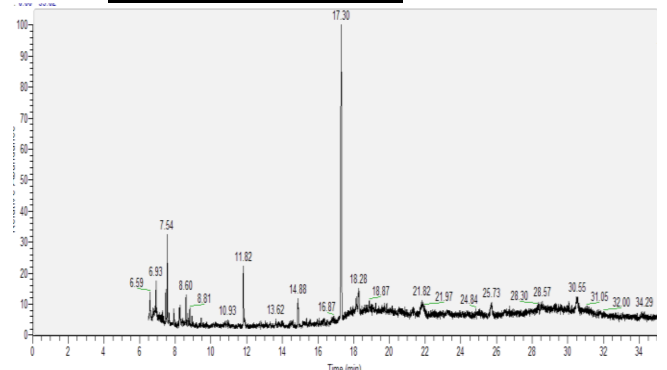

Figure 13. ***GC chromatogram of the fraction 3 and its attribution***

The GC chromatogram revealed the presence of squalene with a considerable probability. Squalene has very rewarding properties and even in research.

E. Antioxidant activity

The use of antioxidant molecules of synthesis is now questioned because of potential toxicological

risks[16]19]. Indeed, polyphenols are natural compounds widely distributed in the plant kingdom that have increasingly importance notably grace to their beneficial effects on health. The role of natural antioxidants attracting more and more interest in the prevention and treatment of cancer, inflammatory and cardiovascular diseases; they are also used as additives in food, pharmaceutical and cosmetics. Scientific research has been developed for the extraction, identification and quantification of these compounds from different sources, such as agricultural and horticultural crops or medicinal plants. Our study aims to evaluate in vitro the antioxidant activity of ethanol extracts according to the trapping method of free radical DPPH.

TABLE 2. RESULT OF CONTROL BY ASCORBIC ACID

Ascorbic acid DPPH								
µg/mL	A1	A2	Med	A1	A2	Med	A blank	I %
2.5	0.234	0.24	0.237	0.515	0.525	0.52	0	54.42308
3.75	0.064	0.072	0.068			0.52	0	86.92308
5	0.036	0.034	0.035			0.52	0	93.26923
7.5	0.03	0.028	0.029			0.52	0	94.42308
10	0.015	0.022	0.0185			0.52	0	96.44231
20	0.015	0.002	0.0085			0.52	0	98.36538

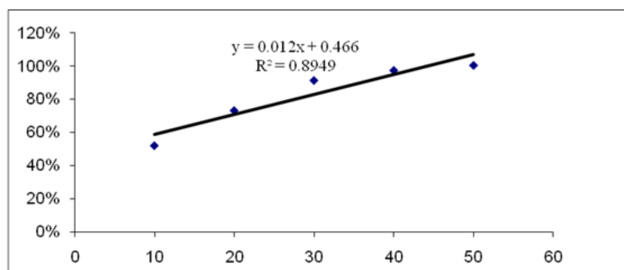

Figure 14. ***Inhibition of DPPH in function of the concentrations of ascorbic acid as control and the corresponding regression line***

The EC50 values determined in mg/mL expressing the effective concentration of the antioxidant extract necessary for trapping and 50% reduction of moles of DPPH dissolved in ethanol.

EC50 (Ascorbic acid) = 2.833 mg/mL

Percent inhibition for each extraction technique is calculated in the following tables and the corresponding regression lines are plotted thereafter to derive EC50.

TABLE 3. THE PERCENTAGES OF INHIBITION OBTAINED BY THE EXTRACTS DERIVED FROM THE MACERATION

C (mg/mL)	A	% of inhibition (Maceration)
0.1	1.112	6.00
0.2	1.102	6.84
0.3	1.084	8.36
0.4	1.058	10.56
0.5	1.003	15.21
A control	1.183	

TABLE 4. THE PERCENTAGES OF INHIBITION OBTAINED BY THE EXTRACTS OF REFLUX METHOD

C (mg/mL)	A	% of inhibition (Reflux)
0.1	0.973	14.34
0.2	0.844	25.70
0.3	0.747	34.24
0.4	0.676	40.49
0.5	0.538	52.64
A control	1.136	

TABLE 5. THE PERCENTAGES OF INHIBITION OBTAINED WITH THE EXTRACTS OF ULTRASOUND METHOD

C (mg/mL)	A	% of inhibition (Ultrasound)
0.1	1.269	6.69
0.2	1.165	14.33
0.3	1.105	18.75
0.4	1.017	25.22
0.5	0.936	31.17
A control	1.36	

TABLE 6. THE PERCENTAGES OF INHIBITION OBTAINED WITH THE EXTRACTS FROM THE SOXHLET METHOD

C (mg/mL)	A	% of inhibition (Soxhlet)
0.1	1.112	8.55
0.2	1.05	13.65
0.3	0.953	21.62
0.4	0.904	25.65
0.5	0.852	29.93
Acontrol	1.216	

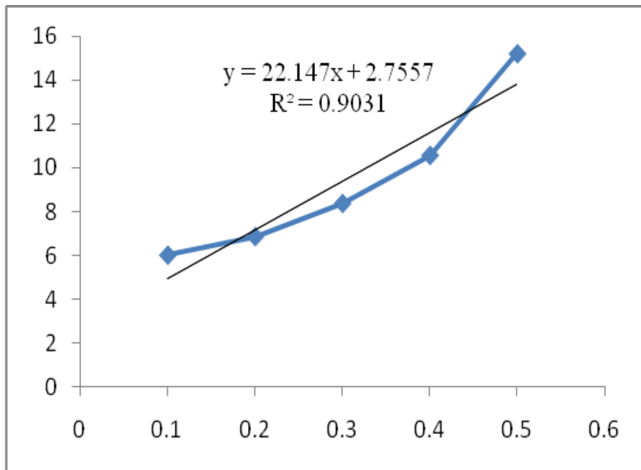

Figure 15. ***% of Inhibition of DPPH in function of the concentrations of the extract by maceration and the corresponding regression line***

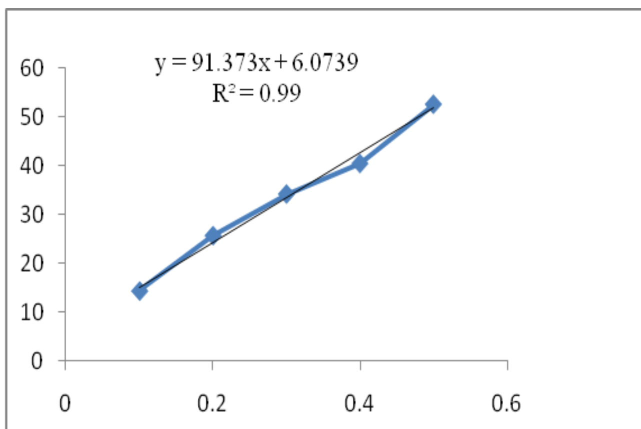

Figure 16. ***% Inhibition of DPPH in function of the concentrations of the extract by reflux and the corresponding regression line***

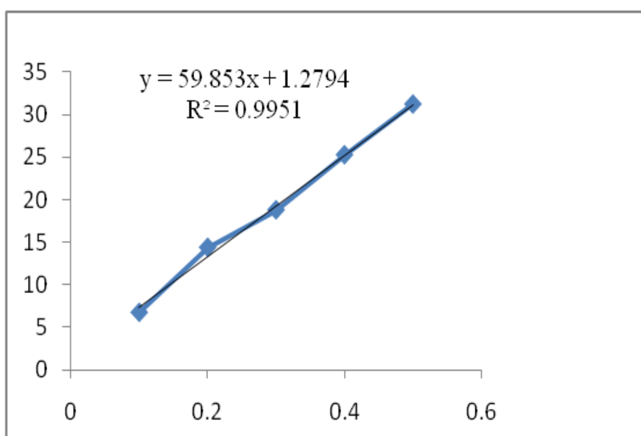

Figure 17. ***% Inhibition of DPPH in function of the concentrations of the extract assisted by ultrasound and the corresponding regression line***

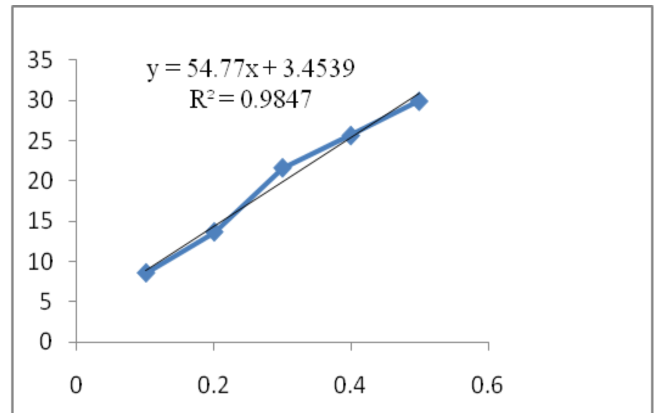

Figure 18. ***% Inhibition of DPPH in function of the concentrations of the extract by the Soxhlet method and the corresponding regression line***

According to the results recorded, the ethanol extracts are provided with a moderate antioxidant power, their EC50 is 1.876 but is relatively lower than that of ascorbic acid with a value of about 2.833 mg/mL. It has been shown that antioxidant molecules such as ascorbic acid, tocopherol, flavonoids and tannins reduce and discolor DPPH due to their ability to give hydrogen. The polyphenols contained in extracts of *E. creticum* are probably responsible for the antioxidant activity of these extracts. This is consistent with the work on the extracts[15]. The values of effective concentrations are calculated and listed in table 7.

TABLE 7. THE EFFECTIVE CONCENTRATIONS OBTAINED FROM DIFFERENT EXTRACTION TECHNIQUES

	Maceration	Reflux	Soxhlet	Ultrasound	Ascorbic acid
EC 50 (mg/mL)	2.133	0.481	0.849	0.814	2.833

- **<u>EC50 comparison with the extracts of other origins:</u>**

In the following sections, we compare the EC50 obtained by extracts that were studied in relation to a history work done for similar studies in other parts of Lebanon[17].

A. BEIRUT:

TABLE 8. THE PERCENTAGE INHIBITION OF ETHANOL EXTRACTS OF *ERYNGIUM CRETICUM* COLLECTED FROM BEIRUT IN FEBRUARY 2014 OBTAINED BY REFLUX (4 H)[18].

Concentrations of EtOH extracts (mg/mL)	Reflux (4 h) 100% EtOH	
	Optical density	(%)
0.1	1.857	6.02
0.2	1.702	13.86
0.3	1.682	14.87
0.4	1.600	19.03
0.5	1.538	22.16

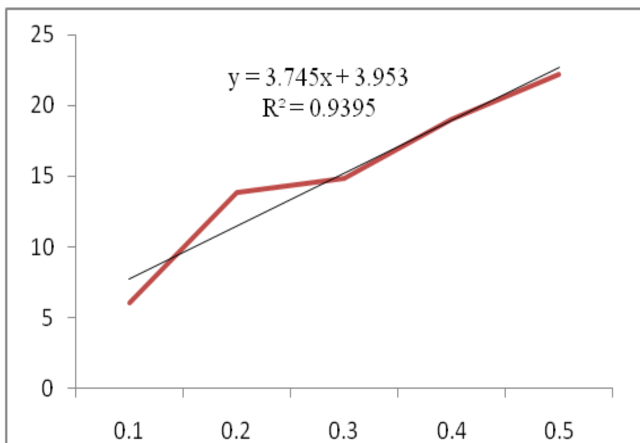

Figure 19. *The regression line of the ethanol extracts of Eryngium creticum collected from Beirut in February 2014 obtained by reflux (4 h)*

The EC50 was calculated for extracts from different regions of Lebanon, the results showed that: EC50 (Beirut) = 1.23 mg/mL > EC50 (Batroun) = 0.481mg/mL. Indeed, the antioxidant capacity of a compound is more elevated as its EC50 is small, so the extracts of Batroun have greater antioxidant activity (2 times greater) than that of Beirut.

B. MOUNT LEBANON

TABLE 9. ANTIOXIDANT ACTIVITY OF THE ETHANOLIC EXTRACTS FROM THE ROWIEST AL BALLUT (FEBRUARY 2014) BY THE DIFFERENT EXTRACTION TECHNIQUES[14].

Technique of extraction	Concentration in mg/mL	Antioxidant activity in %
Maceration	0.1	10.47 $\pm$ 0.044
	0.2	13.1 $\pm$ 0.024
	0.3	19.23 $\pm$ 0.046
	0.4	23.88 $\pm$ 0.046
	0.5	33.53 $\pm$ 0.024
Reflux	0.1	10.43 $\pm$ 0.024
	0.2	17.63 $\pm$ 0.007
	0.3	24.29 $\pm$ 0.028
	0.4	27 $\pm$ 0.025
	0.5	33.16 $\pm$ 0.013
Soxhlet	0.1	14.18 $\pm$ 0.019
	0.2	17.23 $\pm$ 0.013
	0.3	31.23 $\pm$ 0.035
	0.4	41.35 $\pm$ 0.035
	0.5	45.02 $\pm$ 0.029

EC50 (maceration-M.L.) = 0.826 mg / mL < EC50 (Ba.) = 2.133 mg / mL

EC50 (reflux-Be.) = 1.23 mg / mL > EC50 (reflux-M.L.) = 0.801 mg / mL > EC50 (Ba.) = 0.481mg / mL

EC50 (soxhlet-M.L.) = 0.535 mg / mL < EC50 (Ba.) = 0.849 mg / mL

C. South Lebanon

TABLE 10. THE EC50 OBTAINED BY EXTRACTS FROM SOUTHERN LEBANON BETWEEN MARCH AND MAY 2013

	Extracts (UAE)	EC50 (mg/mL)
LEAVES	**Aqueous**	0.22
	Ethanolic	0.18

EC50(S) = 0.18 mg/ml < EC50(Ba) = 0.814 mg/mL

In total, the comparison of the different origins corresponding EC50 is shown in this comparative table to derive the better antioxidant activity.

TABLE 11. A COMPARISON OF EC50 EXTRACTS FROM DIFFERENT BACKGROUNDS AND WITH DIFFERENT TECHNIQUES

Processes / Origins	Reflux	Soxhlet	UAE	Maceration
Batroun	0.481	0.849	0.814	2.133
Beirut	1.23	----	---	----
Mount Lebanon	0.801	0.535	---	0.826
South Lebanon	---	---	0.18	---

By comparing the different results, we note that the extracts from southern Lebanon have the highest activity for the process assisted by ultrasound. On the other hand, for the process reflux, the greatest activity is obtained from the extracts of Batroun. So, the extracts from Beirut have the lowest activity and the extracts from South Lebanon have recorded the greatest activity.

IV. CONCLUSION

The objectives of this study were to extract some active compounds using different extraction techniques. Then, to evaluate the antioxidant activity of the ethanolic extract from both leaves and stems of the Lebanese *Eryngium creticum*. All results indicate that this plant contains bioactive compounds. In addition, it has an important antioxidant activity dependent on the nature of the extract and on its concentration.

On the other hand, several bioactive compounds were isolated and identified from various extracts of the whole plant; these compounds have antimicrobial activity, antioxidant, anti-inflammatory, cosmetic, preventive and anticancer. These results support the use of *Eryngium creticum* for various ailments and it can be used as a source of multi drug resistant in the future.

Acknowledgments

The authors are thankful to Lebanese University for the financial support of this work.

References

[1] G. Tohmé, G., & H.S. Tohmé, Beirut,Lebanon: National Council for Scientific Research, 2007.

[2] K. Marcinkowski, «Handbook of medicinal herbs,» CRC press, pp. 277-278, 2002 .

[3] Abu-Rabia, Herbs as a food and medicine source in Israel, Asia Pac J Cancer Prev 6, pp. 101-107,2005.

[4] MS Ali-Shtayeh, MR Yaghmour, YR Faidi, K Salem, MA Al-Nuri, «Antimicrobial activity of 20 plants used in folkloric medicine in the Palestinian area,» *J Ethnopharmacol,* vol. 6, pp. 265-271, 1998.

[5] O. Said, K. Khalil, S. Fulder, H. Azaizeh, «Ethnopharmacological survey of medicinal herbs in Israel,» *J Ethnopharmacol,* vol. 83, pp. 251-265, 2002.

[6] H. Farhan, F. Malli, H. Rammal, A. Hijazi, A. Bassal, N. Ajouz, B. Badran, «Phytochemical screening and antioxidant activity of Lebanese Eryngium creticum L.,» *Asian Pacific Journal of Tropical Biomedicine,* vol. 2, pp. 1-4, 2012.

[7] H. Rammal, H. Farhan, H., A. Hijazi, K. Ahmad, D. Ahmad, and B. Bassam.«Antioxidant, Cytotoxic Properties and Phytochemical Screening of Two Lebanese Medicinal Plants,» *Int. Res. J. Pharm,* vol. 4, n° 15, p. 132–136, 2013.

[8] S. Zeidan, A. Hijazi, H. Rammal, A. Kobaissi, & B. Badran, «extraction of phenolic compounds and flavonoids from eryngiumcreticum l.By conventional and non- conventional extraction techniques» World Journal of Pharmacy and Pharmaceutical Sciences, vol. 3, n°17, pp. 1889-1898, 2014.

[9] S. Zein, S. Awada, S. Rachidi, A. Hajj, E. Krivoruschko, and H. Kanaan, «Chemical analysis of essential oil from Lebanese wild and cultivated Origanum syriacum l . (Lamiaceae) before and after flowering,» vol. 5, n°13, pp. 379-387, 2011.

[10] B. Chebli, L.M.I. Hassani, & M. Hmamouchi, «Acides gras et polyphénols des graines d' Ononis natrix L. (Fabaceae) de la région d'Agadir,» *Maroc. Acta Botanica Gallica,* vol. 148, n°14, pp. 333-340, 2001.

[11] G. Lopes, C. Sousa, J. Bernardo, P. B. Andrade, P. Valentão, F. Ferreres, and T. Mouga, «Sterol profiles in 18 macroalgae of the portuguese coast,» *J. Phycol,* vol. 47, n°5, pp. 1210-1218, 2011.

[12] Popovici, C., & Saykova, I., «Revue de Génie Industriel Evaluation de l ' activité antioxydant des composés phénoliques par la réactivité avec le radical libre DPPH,» *Revue de Génie Industriel,* vol. 4, pp. 26-39, 2009.

[13] B. Sayed-ahmad, A. Hijazi, H. F. Kazan, and H. Rammal, «Extraction, phytochemical screening , chemical quantification and identification of bioactive compounds from Lebanese Urtica dioica,» vol. 4, n°12, 2014.

[14] S. A. ZEIDEN, «A Comparison between Conventional and Non-Conventional Extraction Techniques for the Determination of Total Amount of Extracts, Total Phenolic Content, Total Flavonoid Content, Total Alkaloids, and Antioxidant Capacity of a Lebanese Plant Eryngium creticum» Lebanese university, 2013.

[15] M. Dammous, H. Farhan, H. Rammal, A. Hijazi, A. Bassal, H. Fayyad-Kazan, Y. Makhour, B. Badran. Chemical composition of Lebanese Eryngium creticum L. International Journal of Sciences, vol 3, n°4, pp. 40-53, 2014.

[16] S. Zeidan, A. Hijazi, H. Rammal, A. Al Bazzal, H. Annan,and A. Al- Ameer N. Al-Rekaby. Determination of bioactive compound content and antioxidant activity of the Lebanese Eryngium Creticum L. Eur. Chem. Bull., vol 4, n°11, pp. 498-502, 2015.

[17] H. Rammal, H. Farhan, N. Jamaleddine, M. El Mestrah, M. Nasser and A. Hijazi. Effects of altitude on the chemical composition and on some biological properties of Lebanese Eryngium creticum L. J. Chem. Pharm. Res., vol 7, n°6, pp.887-893, 2015.

[18] A. Hijazi, D. S. Al Masri, H. Farhan, M. Nasser, H. Rammal, H. Annan. Effect of Different Ethanol Concentrations, Using Different Extraction Techniques, on the Antioxidant Capacity of Lebanese Eryngium creticum. J Pharm Chem Biol Sci , vol 3, n°2, pp.262-271 June-August 2015.

[19] Z. Dirani, R. Makki, H. Rammal, S. Naserddine, A. Hijazi,HF Kazan, M. Nasser,A. Daher and B. Badran. The antioxidant and anti-tumor activities of the lebanese Eryngium Creticum L. IJBPAS, vol 3, n°10, pp. 2199-2222, 2014.