International Journal of Sciences

Research Article Volume 3 - April 2014 (4)

Chemical Composition of Lebanese Eryngium Creticum L.

Mirella Dammous[2], Hussein Farhan[1], Hassan Rammal[1,2], Akram Hijazi[1], Ali Bassal[2], Hussein Fayyad-Kazan[1], Yolla Makhour[1], Bassam Badran[1]

[1]Doctoral School of Science and Technology, Research Platform for Environmental Science (PRASE), Lebanese University, Lebanon
[2]Lebanese University, Faculty of Agronomy and Veterinary sciences, Lebanon

Abstract: This work aimed to purify, identify and quantify some of the bioactive compounds in the aqueous and ethanolic extracts from a Lebanese *Eryngium creticum* L. in addition to evaluate its metal content, particularly trace elements and thus to estimate its antioxidant activity. The phytochemical screening results indicated the presence of different bioactive compounds in both extracts mainly phenolic compounds, alkaloids, glycosides, terpenoids and flavonoids. On the other hand, XRF showed that this plant contains metals such silver, zirconium, nickel, selenium, niobium and molybdenum, iron, calcium, manganese and copper. The results of three in vitro antioxidant methods, DPPH, Ferrozine and H_2O_2 showed a significant antioxidant activity in both ethanolic and aqueous extracts revealing that *E. creticum* is a good source of different antioxidant and bioactive compounds.

Keywords: *Eryngium creticum* L., phytochemical screening, trace elements, antioxidant activity, GC-MS.

1. Introduction

Spices and herbs have been added to foods not only as flavoring agents, but also as folk medicine and food preservatives. Spices and herbs and their constituents are generally recognized to be safe, either because of their traditional use without any documented damaging impact or because of dedicated toxicological studies. Being natural foodstuffs, spices and herbs appeal to many consumers who question the safety of synthetic food additives (Smit et al, 1999).

Presently, there is an increasing interest, both in the industry and in the scientific research in medicinal herbs because of their strong biological properties. These properties are due to many substances, including some vitamins, flavonoids, terpenoids, carotenoids, phytoestrogens, and minerals. Phenolic substances have shown to be the most responsible for the antioxidant activity of plant (Farhan et al., 2012a,b,c; Farhan et al., 2013).

During years, plants have been studied chemically from the viewpoints of biosynthesis of active constituents. The world health organization (WHO, 2002) estimated that 11 % of drugs are obtained exclusively from plants, 10 % of plants species are tested for different biological activities, and out of all discovered drugs, 252 drugs play important role in saving human life from fatal diseases. Arabs countries and more particularly Lebanon are distinguished by a great wealth of plant species especially with medicinal properties. In fact, 2607 wild species of which 92 are endemics can be found in only 10452 km^2. For that, it will be very necessary to conduct recent scientific studies on these endemic plants, especially those used locally as medicine.

Our present study aimed to evaluate the antioxidant capacity of the stems and leaves of the Lebanese *Eryngium creticum* L. using three in vitro antioxidant tests, DPPH, ferrous ions and H_2O_2 and to identify some chemical compounds from this plant.

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. *E. creticum* 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. *E. creticum* showed also an anti-inflammatory property and an anti-microbial activity. It was also used in the treatment of liver diseases, poisoning, anemia and infertility. This plant has showed an antioxidant property by inhibiting the lipid peroxidase in the liver of the rat (Rammal et al., 2009).

2. Materials and Methods

Hassan Rammal (Correspondence)
hasanrammal@hotmail.com
+96170885686

2.1. Plants preparation

2.1.1. Plant collection and preparation of powders

Fresh plant was gathered from south Lebanon (350 m of altitude) on spring season between March and May in 2013. Then, plants were well cleaned and washed with water and then dried at room temperature inside the limit in humid, well-opened to prevent damage. After this period, leaves and stems of the 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.

2.1.2. Ultrasonic assisted extraction (UAE)

UAE involves the application of sound waves of high intensity and high frequency and their interaction with matter. UAE is a potentially useful technology because it does not require complex instruments and is relatively inexpensive. Under the action of ultrasonic solid and liquid particles are vibrated and accelerated, and thus the solutes diffuse rapidly from the solid phase to the solvent. If the ultrasonic intensity is increased in a liquid, then it reaches a point at which the intramolecular forces are not able to maintain intact the molecular structure, so that it breaks down and the bubbles are created, this process is called cavitation. The collapse of bubbles can produce physical, chemical and mechanical effects that lead to the disruption of biological membranes to facilitate the release of extractable compounds, increase the penetration of the solvent into the cells and improve mass transfer (Gupta et al.; 2012).

2.1.3. Ethanolic and aqueous crude extracts

This method was done according to Farhan et al. (2012a). 100 g of green leaves and stems were put in maceration with 500 ml of EtOH (70 %) and 500 ml of distilled water. After 5 hours in the oven at 40 °C, the extraction was carried out for 8 h at room temperature with continuous agitation. After that, extracts were filtered and they have been dried using the rotary evaporator (for the EtOH extraction at 40°C, for the aqueous extraction at 60°C).

2.2. Chemical quantification of secondary metabolites

2.2.1. Total phenolic content (TPC)

The Folin–Ciocalteau reagent method has been used for the estimation of TPC. 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.

2.2.2. Total flavonoids content (TFC)

The aluminum chloride method 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.

2.2.3. Saponin determination

The determination of total saponin was done according to the method used by Farhan et al. (2012c). 1 g of powdered plant has been added to 100 ml of 20 % aqueous ethanol and kept in a flask on stirrer for half hour and then heated over for 4 h at 45 °C with mixing. The mixture was filtered by using Whatman filter paper (0.45 µ) and the residue was again extracted with another 100 ml of 25 % aqueous ethanol. The combined extracts were concentrated by using rotary evaporator at 40 °C to get 40 ml approximately. The concentrate was transferred into separator funnel and extracted twice with 20 ml diethyl ether. The ether layer was discarded while the aqueous layer was kept and then re-extracted with 30 ml n-butanol was added. The n-butanol extracts were washed twice with 10 ml of 5 % aqueous sodium chloride. The remaining solution was evaporated. After evaporation, the samples were dried in the oven at 40°C to a constant weight. The saponin content was calculated using the following formula:

% Saponin = (final weight of sample / initial weight of extracts) × 100

2.2.4. Total alkaloids

The quantification method for alkaloids determinations has been used according to Farhan et al. (2012c). 100 ml of 10 % acetic acid in ethanol was added to 1 g of dry powdered plant and then the extracts were covered and allowed to stand for 4 h. After that, the extracts have been filtrated and concentrated on a water bath to 25 ml of its original volume. The droplets of concentrated ammonium hydroxide were added to the extract until the precipitation the whole solution was allowed to settle, and then the precipitates were washed with dilute ammonium hydroxide and then filtered using Whatman filter paper (N1 0.45µm). The residue was dried in the oven at 40 °C and weighed. The alkaloid content was determined using the following formula:

% Alkaloid = [final weight of the sample / initial weight of the extract] × 100.

2.2.5. Estimation of the proportion of ash

Leaves and stems of *E. creticum* powders have been tested to estimate the proportion of ash using the standard methods of AOAC 923.03 (1980). 1 g was putted in and burned in a furnace burning (muffle

furnace) at 550 °C for 5 h till the obtaining of an gray color of the powders. Then, the residues have been weighted and the percentage of ash has been estimated according the essential dry weight of plant powder.

2.2.6. Determination of total lipids

The estimation of the percentage of total fat (lipids) was done according to AACC method 08-01 (1984). 10 g of powders from leaves and stems of *E. creticum* were added in ultrasound with 200 ml of petroleum ether (40-60 °C) and extracted during 2 h. After that, the solvent was filtered using a Buchner funnel under reduced pressure by Whatman filter paper (0.45 µ), and then an evaporation using a rotary evaporator at 40 °C has been done. Finally, the weight of lipids has been calculated and then the percentage has been determined.

2.2.7. Determination of total protein

The proportion of total protein in leaves and stems of *E. creticum* powders using the method of Lowry et al. (1951). Bovine serum albumin was used as standard and series of dilution in duplicate were prepared from the stock of 1.5 mg/ml (0, 0.3, 0.9, 1.2, and 1.5 mg/ml). For the protein extracts, 2.5 µl from the sample were diluted with 2.5 µl of H_2O (duplicate for each sample). In each well, 25 µl of reagents A' were added (prepared by mixing 1ml of reagent A with 20 µl of reagent S), followed by the addition of 200 µl of reagent B. Incubation in dark for 15 min during which the reaction takes place and a blue color is formed. The absorbance was read by an ELISA reader at 750 nm. The standard curve was plotted and the protein concentration of the samples was determined.

2.2.8. Determination of minerals

The microwave digestion method was used to determine the amount of seven elements (Zn, Mn, Ca, Cu, Fe, Mo and Nb) in dried leaves and stems of *E. creticum*. This method provides for the acid digestion of the dried plant tissue in a closed vessel device using temperature control microwave heating (Milstone Ethos DG-AG-02) for the metal determination by spectroscopic methods. 0.5 g of dried plant samples were placed into microwave digestion inside TFM vessels and 8 ml of a freshly prepared mixture of concentrated HNO_3 (65%)–H_2O_2 (30%) (7:1, v/v) were added to each vessels and stood for 10 min.

The microwave digestion system consists of two steps, 10 min for every step on 200 ˚C and the microwave power up to 1000 Watt. The samples have been left for one day and resulting solutions after cooling diluted and complete the volume to 50 ml with ultra-pure water and then filtrated by 0.25 µ filter units to taken the solutions which can be used to determine the metal with atomic absorbance spectrometry (AAS).

2.2.9. Tannins determination

The method of Dalaly & Hassan (1987) has been used to determine the amount of tannin. 0.5 g from leaves and stems of *E. creticum* powders has been taken. Each plant parts extracted by 75 ml of distilled water in water bath boiling for 1 h and after that placed the mixture in centrifuge 2000 rpm for 20 min. The supernatants shift to flask and the volume was completed to 100 ml with distilled water. Added to supernatants 20 ml of lead acetate 4 % and set in shaker for 1 h. These solutions were filtered by whatman 0.45 filter paper and the residues have been moved to ceramic container and placed in oven at 105˚C for 1 h and the samples weight to give T1 and again put the samples in muffle furnace at 550 ˚C for 2 h and then weight the samples again to give T2. The percentage of tannins was calculated on dry weight of plant samples according the equations:

% Tannins = (T1-T2 /dry weight) x 100

2.3. Evaluation of the antioxidant activity

2.3.1. DPPH radical scavenging activity

The method of Farhan et al. (2012a) has been used for the scavenging ability of DPPH antioxidant test. 1 ml of different concentrations of diluted extracts of the plant parts in ethanol was added to 1ml of DPPH (0.15 mM in ethanol) and at the same time, a control consisting on 1ml DPPH with 1ml ethanol 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 ascorbic acid was used as a positive control and the ethanol was used as blank. The DPPH scavenging ability of plant extracts was calculated using the following equation:

% *Scavenging* activity = [(Abs control − Abs sample)]/ (Abs control)] ×100

The Abs control is the absorbance of DPPH + ethanol; Abs sample is the absorbance of DPPH radical + sample. Also, three controls have been prepared.

2.3.2. Scavenging activity of hydrogen peroxide (H_2O_2) radical

The H_2O_2 scavenging of the extracts of leaves and stems of the different plants was determined according to the method of Farhan et al. (2012b). A solution of H_2O_2 (40 mM) was prepared in PBS (pH 7.4) and concentration was determined spectrophotometrically (Gene Quant 1300 UV-Vis) at 230 nm. Different concentrations of extracts from stems and leaves of this plant in distilled water were added to a H_2O_2 solution (0.6 ml, 40 mM) and the absorbance of H_2O_2 at 230 nm was determined after 10 min, a blank solution containing phosphate buffer

without H_2O_2. Ascorbic acid was used as stander reference.

The percentage scavenging of H_2O_2 was calculated using the following equation:
***% Scavenged* [H_2O_2] = [(Abs control – Abs sample) / Abs control] × 100.**

2.3.3. Chelating effects on ferrous ions
The method of Farhan et al. (2012c) has been used to estimate the chelating effect on ferrous ions. 0.5 ml of various concentrations of all extracts was mixed with 0.5 ml of $FeSO_4$ (0.12 mM), and with 0.5 ml of Ferrozine (0.6 mM). The mixtures were allowed to stand for 10 min at room temperature. After incubation, the absorbance was measured by Gene Quant 1300 UV- Vis spectrophotometrically at 562 nm. Ultra-pure water of sample solution was used as a control without extracts, ultra-pure water was used as a blank. EDTA-Na_2 was used as reference standard. All measurements were performed in Triplicate. The Ferrozine solution (3-[2-Pyridyl]-5,6-diphenyl-1,2,4-triazine-4,4´-disulfonic acid Na-salt) (0.6 mM) was prepared in Ultra-pure water and stored in the dark at room temperature. The ability of the sample to chelate ferrous ion was calculated relative to the control (consisting of iron and Ferrozine only) using the formula:
Ferrous ion-chelating ability (%) = [(A control − A sample) / A control] ×100

2.4. Purification and identification
The various extracts were subjected to a pre-separation column chromatography on silica gel. The solvents used in this step are identified based on their ability to separate the extract on an analytical TLC plate. Then, a second purification step is necessary where each eluted fraction was separated on a preparative TLC plate. The fractions are then analyzed by GC-MS.

2.4.1. GC-MS analysis
The GC agilent analysis was done with Supelco Analytical, fused silica capillary column SP 2380 with 60m*0.25mm*0.25 um film thickness.
Entry temperature: 250 ° C
Injection program:
• Initial T: 50 ° C
• 50 ° C -60 ° C (10 ° C / min) / 2 min remaining
• 60 ° C-80 ° C (20 ° C / min) / 2 min remaining
• 80 ° C -90 ° C (10 ° C / min) / 2 min remaining
• 90 ° C -120 ° C (3 ° C / min) / 3 min remaining
• 120 ° C -150 ° C (5 ° C / min) / 5 min remaining
• 150 ° C -250 ° C (10 ° C / min) / 15 min remaining
• 250 ° C -300 ° C (20 ° C / min) / 5min remaining
Flow of helium (carrier gas) 1mL/min
The GC is coupled with MS and the temperature of transfer line is 280 ° C.
Fashion: FAB, positive, full screen.
The interpretation of mass spectra of GC-MS is performed after "The National Institute of Standards and Technology" (NIST) with more than 62.000 motifs. The name, molecular weight and structure of the compound are then recognized.

2.5. Statistical analysis
All analyses were carried out in triplicates except total protein was in duplicates. The result of scavenger activity, TPC and TFC were performed from the averages of all samples reading mean ± SD Used Excel 2003. Also the results of antioxidants have been done by SPSS version 16 using Student's t-test ($P < 0.05$).

3. Results and discussion
3.1. Active contents
As shown in Table 1, the percentage of active compounds differs between leaves and stems of *E. creticum.* The amount of total saponin, lipid and alkaloid were higher in leaves than in the stems. On the other hand, the amount of total protein was higher in the EtOH than in the aqueous extraction. Moreover, the amount of total tannins was negligible in these two parts.

The ash content represents the percentage of mineral elements in plants, where a high level corresponds to an increased percentage of mineral elements. In Lebanon, the soil is considered to be rich and fertile so there is an increase in the rate of mineral elements in the plants and therefore an increase in the percentage of ash.

Table 1: Percentage of active contents in leaves and stems of *Eryngium creticum*

	LEAVES		STEMS	
Total saponin	10.76		7.84	
Total alkaloid	5.66		1.49	
Total ash	22.1		18.07	
Total lipid	4.104		0.846	
Total protein	EtOH	Aqueous	EtOH	Aqueous
	13.3	11.98	9.76	4.3

3.2. Total phenolic and total flavonoid contents
The TPC and TFC in the leaves and stems of *E. creticum* were evaluated. The absorbance of these extracts was measured for two different concentrations. As shown in Table 2, aqueous and EtOH extracts from both leaves and stems of this

plant were found to contain high amounts of TPC and TFC in favor of the EtOH extract. Moreover, the amounts of TPC and TFC in the leaves were higher than in the stems.

Table 2: TPC and TFC in leaves and stems of *E. creticum* (mg GAE/g dry weight)

	LEAVES		STEMS	
	Aqueous	**EtOH**	**Aqueous**	**EtOH**
TPC	59.2 ± 0.07	253 ± 0.031	47.5 ± 0.028	230 ± 0.043
TFC	195.2 ± 0.063	729±0.023	116.1±0.031	258.9 ± 0.041

Values are the average of triplicate experiments and values expressed as mean ± SD

3.3. Minerals and heavy metals

E. creticum contains various concentrations of trace elements and heavy metals. There are two major reasons to examine the levels of toxic metals in medicinal plants (De Smet, 1992): The first reason is the increase in the contamination of the general environment with toxic metals. Heavy metal contents in spices and medicinal plants depend on climatic factors, plant species, air pollution, and other environmental factors (Sovljanski et al., 1989). The second reason, exotic herbal remedies, particularly those of Asian origin, were repeatedly reported to contain toxic levels of heavy metals or arsenic.

Normal levels of heavy metals are respectively between 20-200 mg/kg dry mass for iron, 1-25 mg/kg dry weight for copper and 0.5-300 mg/kg dry weight for zinc (Walsh, 1971). Comparing these levels with those presented in Table 3, we can see that our sample contains a normal and healthy level of heavy metals. In fact, we found that *E. creticum* is a plant which does not have a tolerance to heavy metals. These characteristics have to be considered as a good bio-indicator in science and environmental studies. Our results demonstrated also that the amount of trace elements differs between the leaves and stems of the studied plant.

The results obtained using microwave digestion showed that both leaves and stems contain different amounts of Fe, Ca, Mn and Cu (Table 3). These elements were higher in leaves than in stems and the Ca was found at higher concentration (321.06 mg/kg) in the leaves than in stems (46.43 mg/kg) comparing to other trace elements. These results show that *E. creticum* has a good nutritional value.

Table 3: The minerals found in the leaves and stems of *E. creticum* **(**mg/kg dry weight)

	LEAVES	**STEMS**
Iron (Fe)	42.9401 ± 0.0002	30.2109 ± 0.0008
Manganese (Mn)	18.3860 ± 0.0002	6.0438 ± 0.0004
Calcium (Ca)	321.0663 ± 0.0001	46.4348 ± 0.0002
Copper (Cu)	2.3531 ± 0.0001	0.83809 ± 0.0001

Values are the average of triplicate experiments and values expressed as mean ± SD

After the analysis of 1 g of powdered stems and leaves of *E. creticum*, the results of fluorescence spectrometry X-rays are shown in Figure 1 and 2 respectively. This Lebanese plant contains various forms of metals particularly niobium and molybdenum. These two metals are of great importance in medicine. In humans, four enzymes depend on molybdenum: sulfite oxidase, xanthine oxidoreductase, aldehyde oxidase, and mitochondrial amidoxime reductase (Mendel, 2009). People severely deficient in molybdenum have poorly functioning sulfite oxidase and are prone to toxic reactions to sulfites in foods (Cohen et al., 1973) and some niobium alloys are physiologically inert and thus hypoallergenic. For this reason, niobium is found in many medical devices such as pacemakers (Mallella et al., 2004).

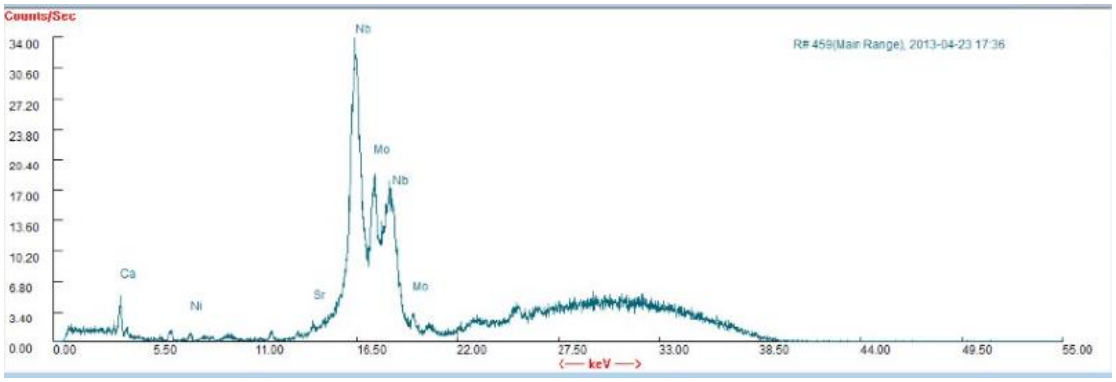

Fig. 1: X-ray fluorescence spectrum of the leaves of *Eryngium creticum*.

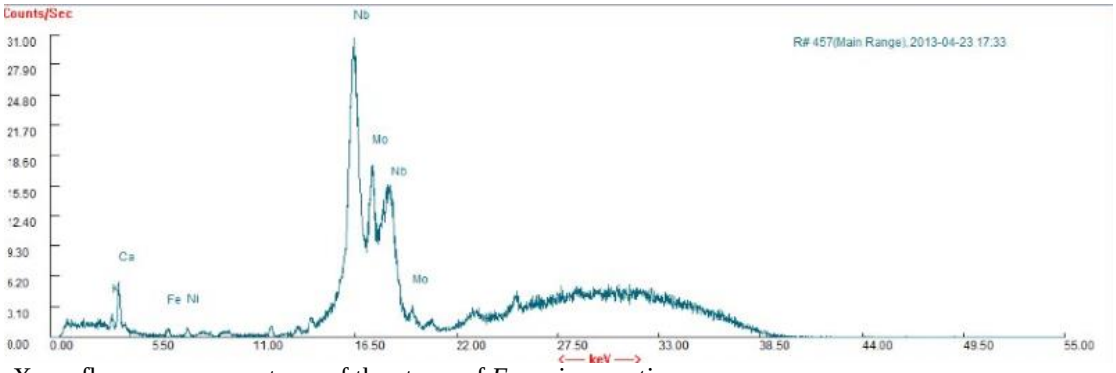

Fig. 2: X-ray fluorescence spectrum of the stems of *Eryngium creticum*.

The percentage of heavy metals of antimony in leaves and stems was 1.61 and 1.37 respectively. The percentage of lead was found to be 0.5 and 0.4 in leaves and stems respectively. Moreover, *E. creticum* was found to contain different amounts of other trace element as shown in Table 4.

Table 4: **The percentage of minerals in stems and leaves of *E.* creticum by XRF.**

	Stems (%)	Leaves (%)
Silver (Ag)	0.6	0.4
Molybdenum (Mo)	1.4	1.1
Zirconium (Zr)	0.4	0.4
Nickel (Ni)	0.7	0.6
Selenium (Se)	0.09	0.08
Niobium (Nb)	2.8	2.3

3.4. Antioxidant activity

A number of reports on flavonoids, triterpenoids, and polyphenols indicated that they possess antioxidant and free radical scavenging activity (Frankel, 1995). These phyto-constituents may exert multiple biological effects against tumors, heart disease, AIDS, and different pathologies due to their free radical scavenging activities. Realizing these facts, our study was carried out to evaluate the antioxidant power of the EtOH and aqueous extracts from stems and leaves of *E. creticum.* An easy, rapid and sensitive method for the antioxidant screening of plant extracts is the free radical scavenging assay using DPPH assay. In the presence of an antioxidant, DPPH radical obtains one or more electrons and the absorbance decreases (Koleva et al., 2002). Our results showed that EtOH extract from both leaves and stems of *E. creticum* has a higher antioxidant activity at different studied concentrations as shown in Figures 3 and 4. On the other side, 5 mg/ml of the aqueous and EtOH extracts from the stems of *E. creticum* significantly increased the % of scavenger activity by 75 % and 79 % respectively as shown in Figure 3.

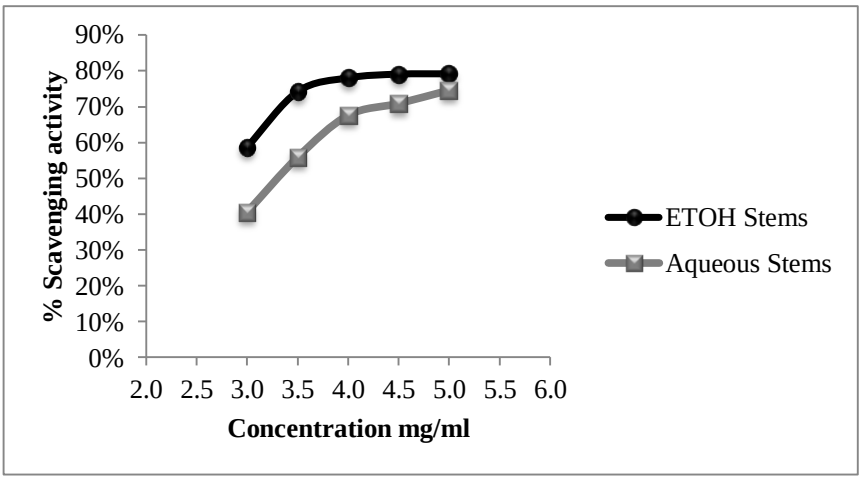

Fig. 3: Positive correlation between the concentrations of EtOH and aqueous extracts from stems of *E. creticum* and the DPPH test.

Also, the antioxidant activity of the EtOH and aqueous extracts from the leaves of *E. creticum* was evaluated using the DPPH assay. The obtained results showed that 0.5 mg/ml of the aqueous and EtOH extracts from the leaves of *E. creticum* significantly increased the % of scavenger activity by 79 % and 81 % respectively as shown in Figure 4.

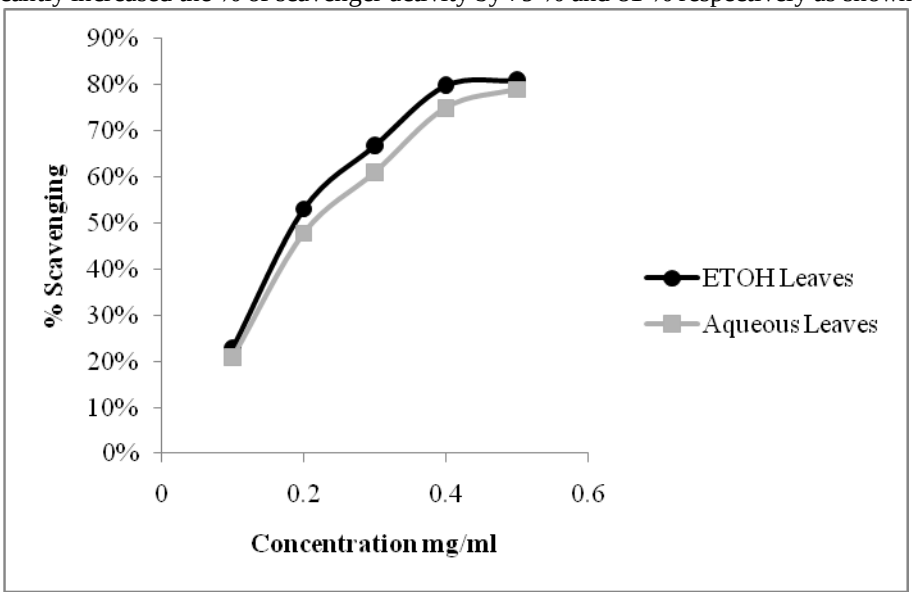

Fig. 4: Positive correlation between the concentration of EtOH and aqueous extracts from leaves of *E. creticum* and the DPPH test.

In order to confirm the antioxidant power of these extracts from the two parts of the studied plant, another well-known in vitro test, the H_2O_2 test was used. Hydrogen peroxide is an important reactive oxygen species because of its ability to penetrate biological membranes. However, it may be toxic if converted to hydroxyl radical in the cell. Scavenging of H_2O_2 by the plant extracts may be attributed to their phenolics, which donate electron to H_2O_2, thus reducing it to water. Our results demonstrated that extracts from both leaves and stems of *E. creticum* were able to scavenge H_2O_2 in a concentration dependent manner. Both EtOH and aqueous extracts from the leaves of *E. creticum* were able to scavenge H_2O_2 in a concentration dependent manner. The % of scavenger activity of 5 mg/ml of leaves was 82 % and 76 % for the EtOH and aqueous extracts respectively as shown in Figure 5.

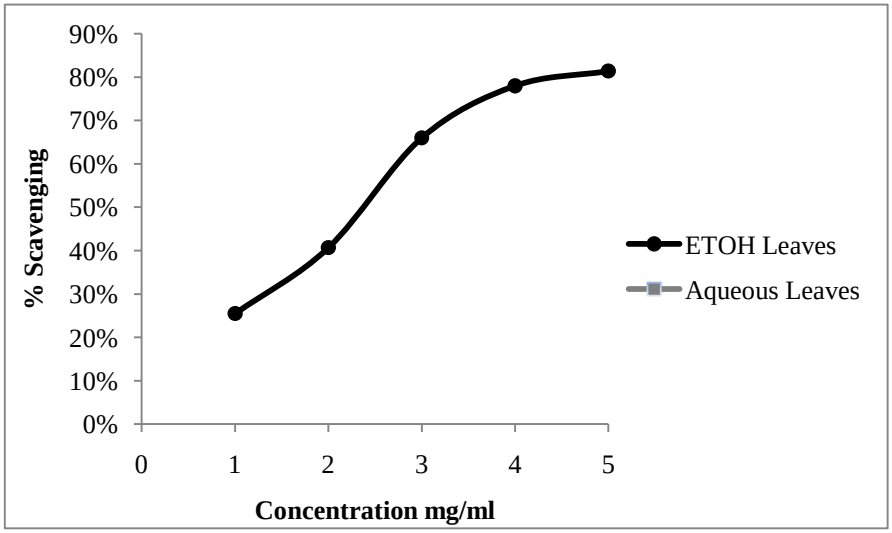

Fig. 5: Positive correlation between the concentration of EtOH and aqueous extracts from leaves of *E. creticum* and the H_2O_2 test.

Therefore, the % of scavenger activity of 25 mg/ml of the stems of *E. creticum* was 64 % and 74 % for the aqueous and EtOH extracts respectively as shown in Figure 6 and 7 respectively.

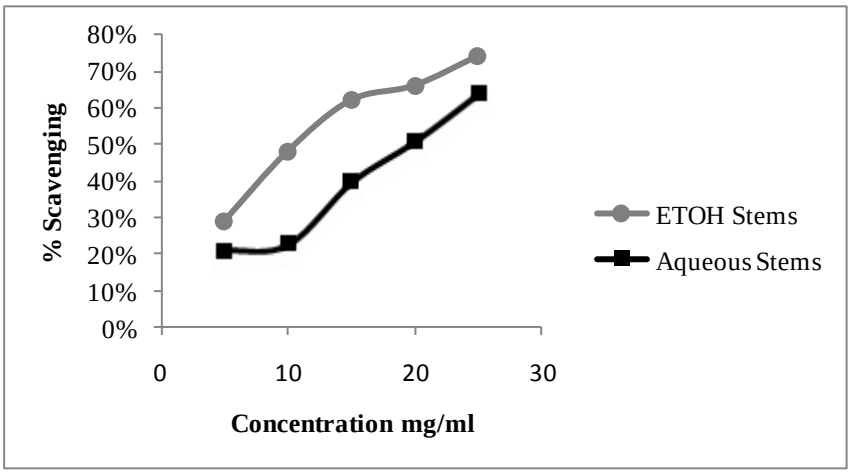

Fig. 6: Positive correlation between the concentration of EtOH and aqueous extracts from stems of *E. creticum* and the H_2O_2 test.

Antioxidants inhibit interaction between metal and lipid through formation of insoluble metal complexes with ferrous ion (Hsu et al., 2003). The iron-chelating capacity test measures the ability of antioxidants to compete with ferrozine in chelating ferrous ion (Elmastas et al., 2006).

Figure 7 showed the metal chelating effect of two extracts from *E. creticum* leaves. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of samples possessing chelating activity, the formation of complexes is decreased. Therefore, measurement of the rate of color reduction helps to estimate the chelating activity of the samples.

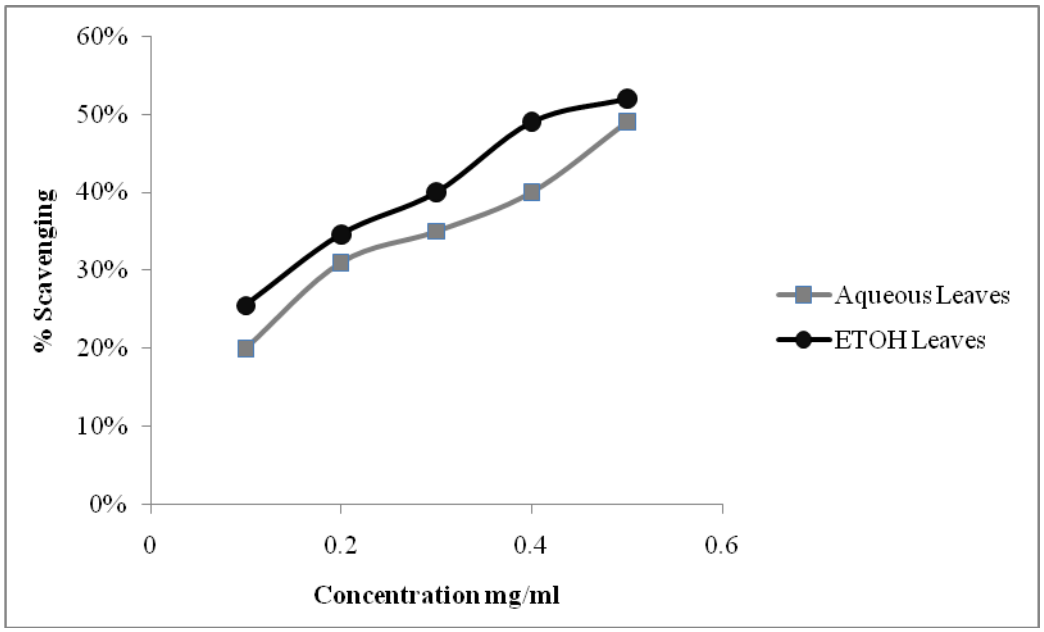

Fig. 7: Positive correlation between the concentration of EtOH and aqueous extracts from leaves of *Eryngium creticum* and the Ferrozine test.

As shown in Figure 7, the chelating capacity of the extract increases with concentration. The order of metal chelating effect of extracts at 0.5 mg/ml from the leaves was: Aqueous extract (49 %) < EtOH extract (52 %).

Among the two extracts from the leaves, EtOH extract showed higher chelating activity compared to the aqueous extract from the leaves of this plant.

Experimentally, there are no results for the stems antioxidant.

All of these results showed that *E. creticum* possess high antioxidant activity and by consequence, it can be considered as a good source of natural products that may be employed in the treatment of different diseases associated with the oxidative stress.

In order to determine the concentration of the extracts at which 50 % of the initial DPPH, H_2O_2 and

Ferrozine were decreased, the IC_{50} was studied. Our results showed that the IC_{50} was different between leaves and stems of the studied plant. As shown in Table 5, aqueous extract from both leaves and stems showed an IC_{50} higher than that of stems for the three in vitro tests. For the EtOH extract, the IC_{50} of stems was 3 mg, while that of leaves was 0.18 mg for the DPPH test. At the same time, the IC_{50} of H_2O_2 was 12 mg for the stems and 2.4 mg for the leaves. On the other hand, the IC_{50} of iron chelating was 0.4 mg for the EtOH leaves extract and 0.5 mg for the aqueous leaves extracts.

Table 5: IC_{50} values of stems and leaves of *E. creticum* for the DPPH, iron chelating and H_2O_2 tests.

	Extracts	IC_{50} (mg/ml)		
		DPPH	H_2O_2	Fe
Leaves	**Aqueous**	0.22	2.6	0.5
	EtOH	0.18	2.4	0.4
Stems	**Aqueous**	3.2	20	-
	EtOH	3	12	-

3.5. Identification of bioactive compounds in total lipid

The first step in the analysis of the hexane extract was pre-purified by column chromatography on silica gel. This step eluted three fractions (F1, F2, and F3). A second step requires a thin layer chromatography (preparative TLC) which was applied to each eluted fraction: F1 gave eight fractions (F1E1, F1E2, F1E3 ....) and F2 gave six fractions and F3 gave seven fractions.

Several fractions were then analysed by GC-MS.

3.5.1. Determination of the structure F1E4

Analysis by GC / MS was used to separate and identify the compounds present in the hexane extract. For the first fraction F1E4, at least 5 majority peaks are observed in the chromatogram (Figure 8). Each peak is identified by comparing the MS spectrum obtained by the one proposed by the library (NIST NO. 02).

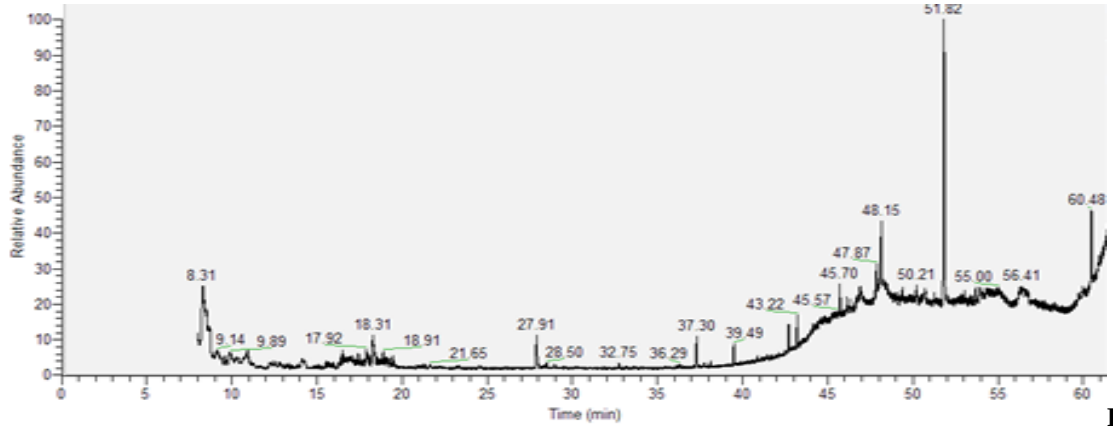

Fig. 8: The chromatogram of the fraction F1E4.

According to the MS spectrum corresponding to the retention time of 8.31 min, a probability of 75 % confirms the presence of acetic acid phenylmethoxyamino trimethyl ester (Figure 9).

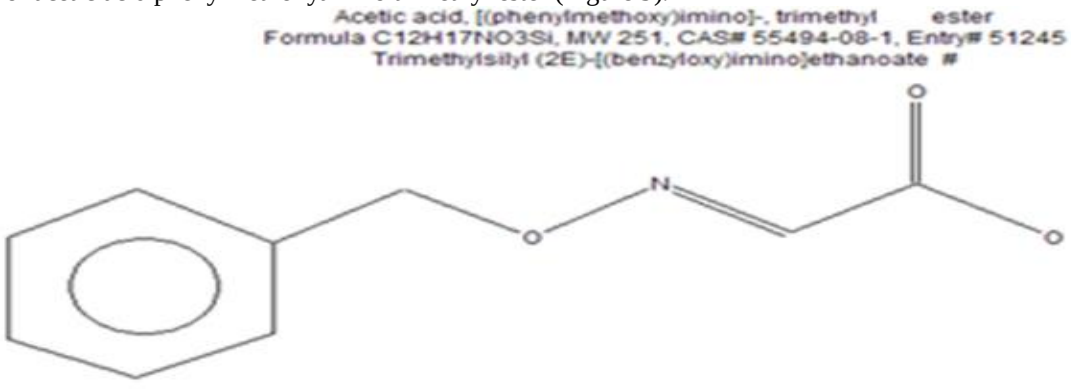

Fig. 9: The chemical structure of acetic acid phenylmethoxyamino trimethyl ester.

For the retention time 60.48 min, a probability of 10 % confirms the presence of Phenol 2,2-methylenebis (6-1.1-dimethylethyul)-4-mthyl (Figure 10).

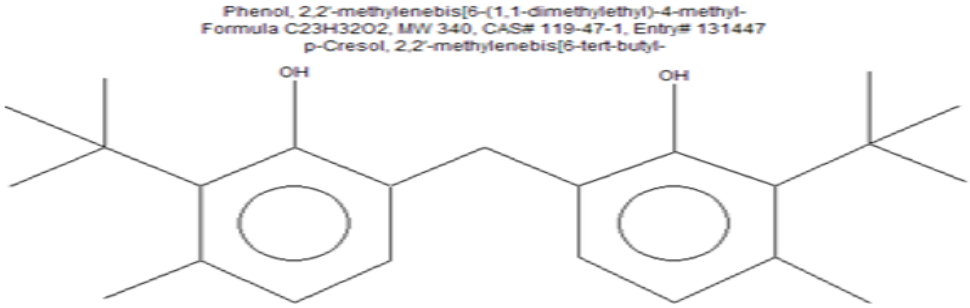

Fig. 10: Chemical structure of Phenol 2,2-methylenebis (6-1.1-dimethylethyul)-4-methyl.

3.5.2. Determination of the structure F1E6

Analysis by GC/MS of the fraction F1E6 shows the presence of at least five peaks (Figure 11).

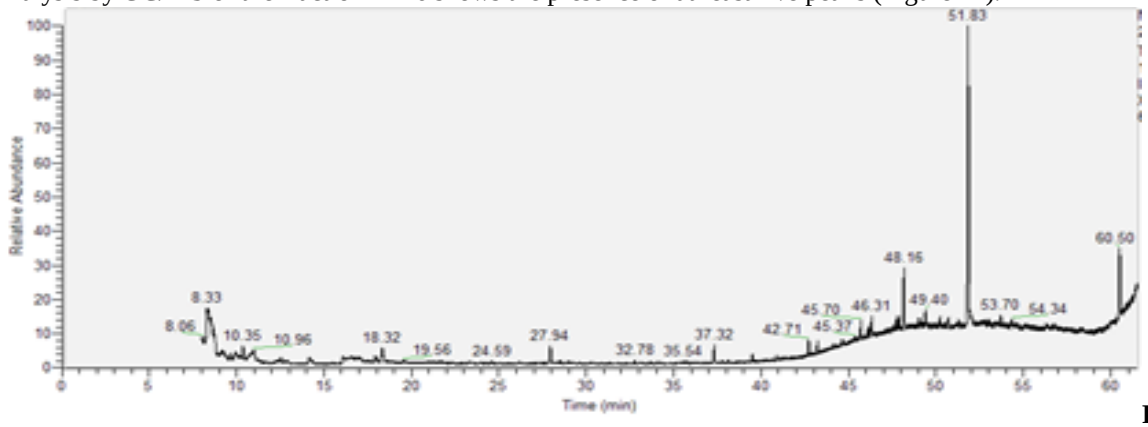

Fig. 11: The chromatogram of the fraction F1E6.

At a retention time of 8.33 min, a peak is obtained. A probability of superimposition of the spectrum was 75 % and that confirms the presence of Methyl 2-O-benzyl-d-arabinoside (Figure 12).

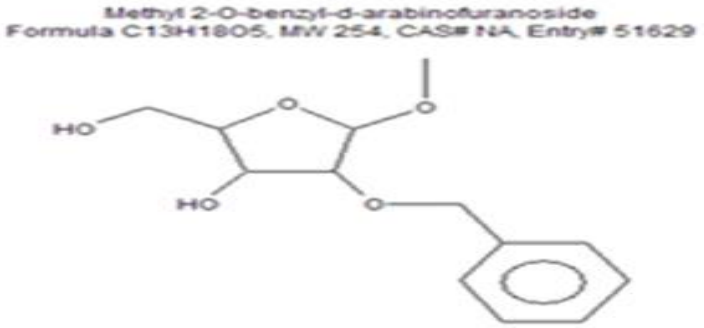

Fig. 12: Chemical structure of Methyl 2-O-benzyl-d-arabinoside**.**

Figure 13 shows that at the retention time 48.16 min, the probability of 23 % confirms the presence of undecanoic acid 1.1-bromo methylester.

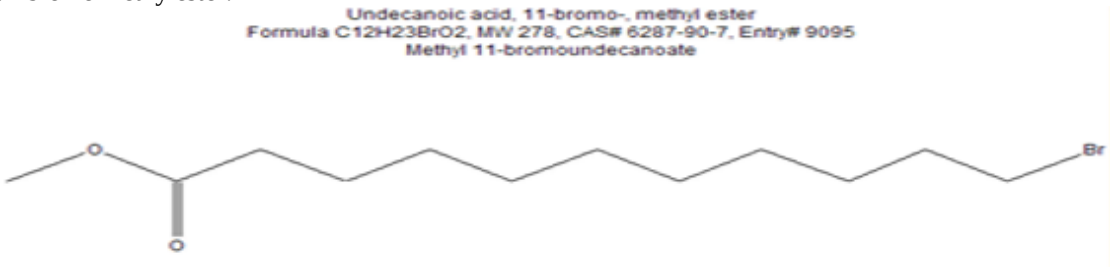

Fig. 13: Chemical structure of undecanoic acid 1.1-bromo methylester.

3.5.3. Determination of the structure F2E3

Seven majority peaks were observed in the chromatogram of GC / MS of the fraction F2E3 as shown in the Figure 14.

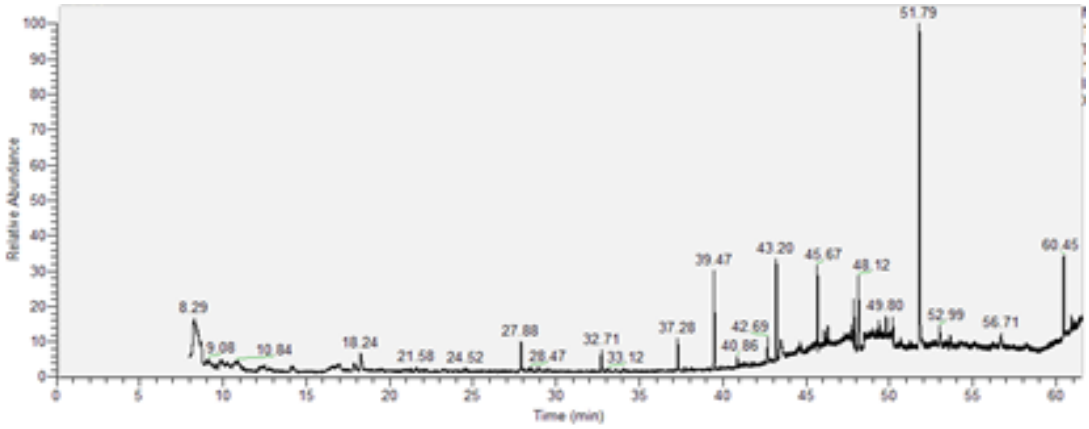

Fig. 14: The chromatogram of the fraction F2E3.

According to the corresponding retention time 39.4 min MS spectrum, the probability of superposition of the spectrum obtained and spectrum-book is 20 %, confirming the presence of 2.6-dihydroacetophenone bis(trimethyl) ether (Figure 15).

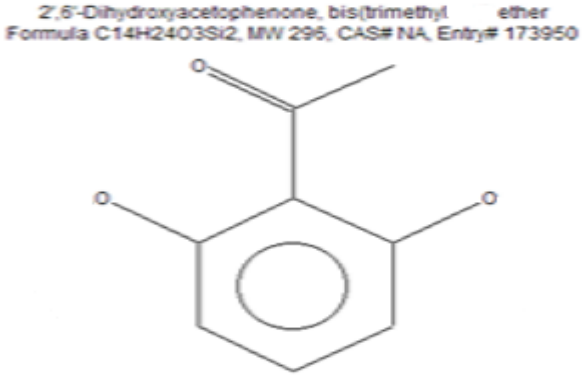

Fig. 15: Chemical structure of 2.6-Dihydroacetophenone bis(trimethyl) ether.

For the retention time 8.29 min, the probability is 72 %, confirming the presence of Benzyloxymethylimine (Figure 16).

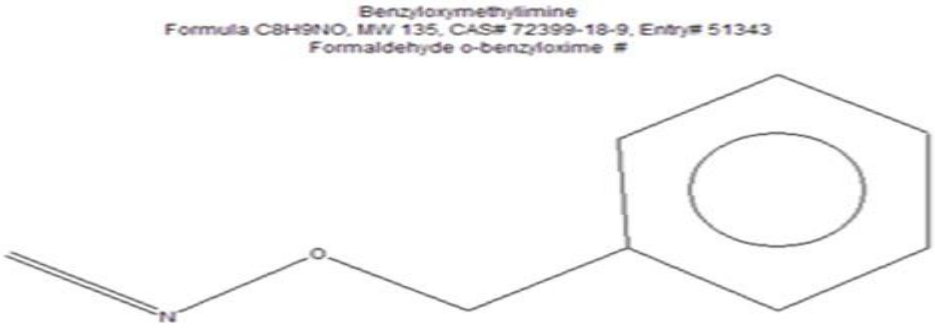

Fig. 16: Chemical structure of Benzyloxymethylimine**.**

3.5.4. Determination of the structure F2E7

Analysis by GC / MS of the fraction F2E7 shows the presence of at least four peaks in the majority chromatogram as shown in Figure 17.

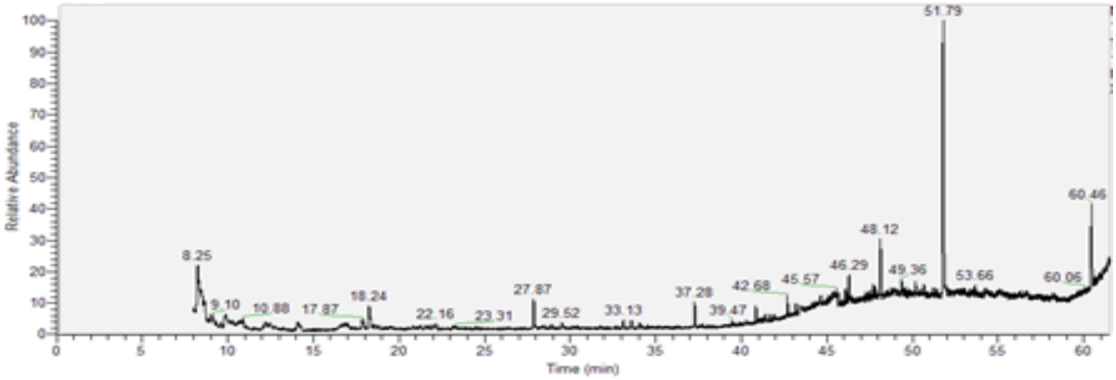

Fig. 17: The chromatogram of the fraction F2E7.

At 51.79 min, a peak is obtained, a probability of superimposition of the spectrum and spectrum obtained by library was 25 % confirming the presence of octadecanoic acid methyl ester (Figure 18).

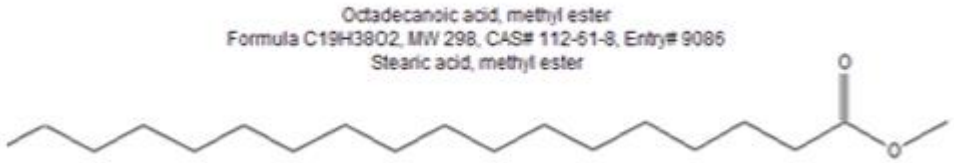

Fig. 18: Chemical structure of octadecanoic acid methyl ester.

For a probability of 75 % at 8.25 min, the presence of 5-Heptyn-3-ol, 2-benzytoxy-7-methoxy-1-(t-butyldimethyl) was observed as shown in Figure 19.

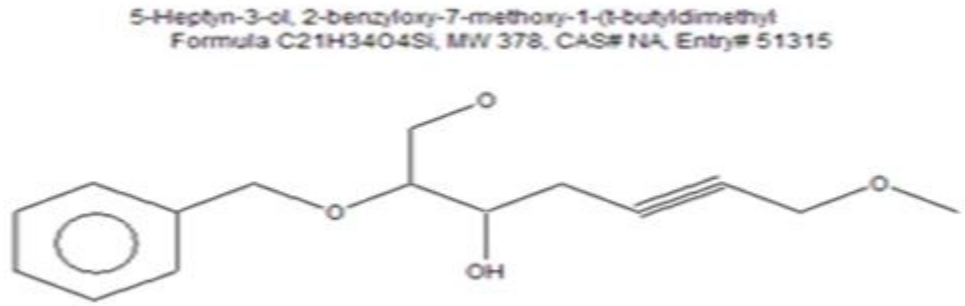

Fig. 19: Chemical structure of 5-Heptyn-3-ol,2-benzytoxy-7-methoxy-1-(t-butyldimethyl).

3.5.5. Determination of the structure F3E3

Four majority peaks were observed in the chromatogram of GC / MS for the E3F3 fraction (Figure 20).

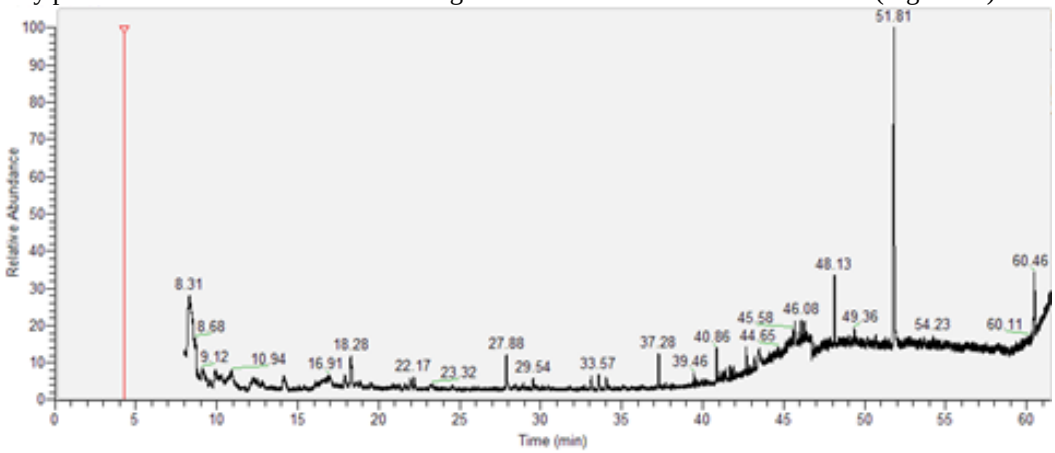

Fig. 20: The chromatogram of the fraction F3E3.

According to the corresponding retention time 40.65 min in the MS spectrum, the probability of superposition of the spectrum obtained and spectrum-book is 40 %, confirming the presence of 9,12,15-Octadecatrinoic acid, 2((trimethyloxy) 1(trimethyloxymethyl) ethyl ester (Figure 21).

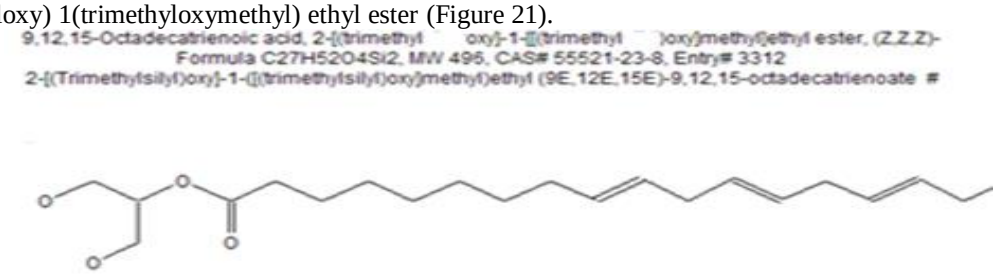

Fig. 21: Chemical structure of 9.12.15-Octadecatrinoic acid, 2((trimethyloxy) 1-(trimethyloxymethyl) ethyl ester.

And for the retention time 49 min, the probability of superposition of the spectrum obtained and spectrum-book is 11 %, confirming the presence of 1.2- benzisothiazol-3-amine as shown in Figure 22.

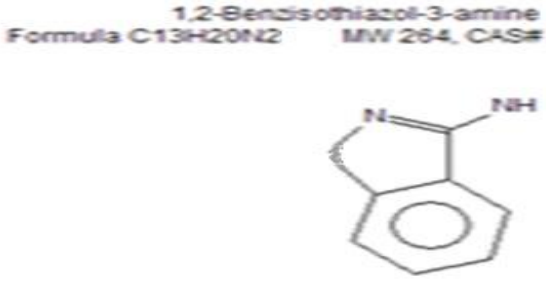

Fig. 22: Chemical structure of 1.2- benzisothiazol-3-amine

3.5.6. Determination of the structure F3E7

Analysis by GC / MS of the fraction F3E7 shows the presence of at least six peaks in the majority chromatogram Figure 23.

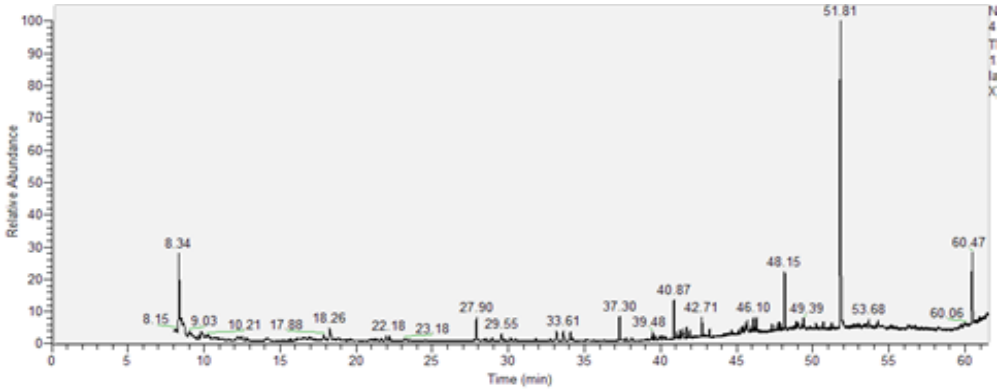

Fig. 23: The chromatogram of the fraction F3E7.

For the retention time 40.87 min, a peak is obtained, a probability of superimposition of the spectrum and spectrum obtained-library was 37.03 % confirming the presence of Phenol 2.4-bis(1.1dimethylethyl) (Figure 24).

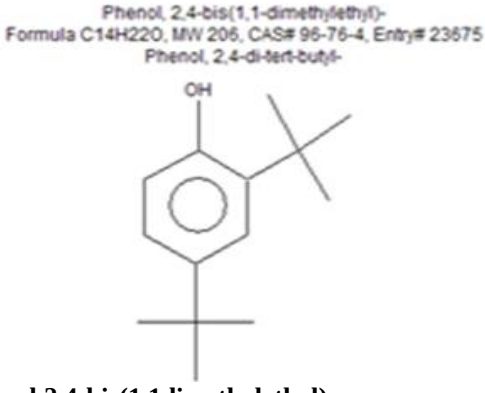

Fig. 24: **Chemical structure of Phenol 2.4-bis(1.1dimethylethyl)**

4. Conclusion

Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, pharmaceutical intermediates and chemical entities for synthetic drugs. All our obtained results demonstrated that in the Lebanese plant *Eryngium creticum* the content of active molecules was different between stems and leaves.

All the obtained results show that this plant contains high levels of minerals and bioactive compounds. In addition, it has an important antioxidant activity dependent on the nature of the extract and concentration, and it contains normal levels of heavy metals, thus there are no toxic side effects that may be associated with its use.

On the other hand, the presence of high amount of proteins indicates that this plant may be considered as a good source of natural products necessary for our daily life.

In a future study, the evaluation of the amount of different vitamins should be done in order to complete and to estimate the nutritional value of the Lebanese plant.

References

1) American Association of Cereal Chemists (AACC) (1984). Method 08-01. The Association St. Paul MN. Approved Methods of the AACC, 8th Edition. Antioxidant activity of algal extracts on lipid peroxidation. *Journal of Medical Science*, 3, 87-98.
2) Association of official Analytical chemists (AOAC) (1980). Official Methods of analysis. 13th ed. Washington D.C. 376-384.
3) Cohen, H.J., Drew, R.T., Johnson, J.L., Rajagopalan, K.V. (1973). Molecular Basis of the Biological Function of Molybdenum. The Relationship between Sulfite Oxidase and the Acute Toxicity of Bisulfite and SO_2. *Proceedings of the National Academy of Sciences of the United States of America* 70 (12 Pt 1–2), 3655–3659.
4) Dalaly, B.A. & Hassan, S. (1987). Food Analysis. Department of Book House for printing and publishing. University of Mosul Arabic Version. Iraq
5) De Smet, P.A.G.M. (1992). Adverse Effects of Herbal Drugs; Springer-Verlag: Berlin; ISBN 3-540-53100-9. Giron, H. C. At. Absorpt. Newsl. 1973, 12, 28.
6) Elmastas, M., Gulcin Isildak, O., Kufrevioglu, O.I., Ibaoglu, K., Aboul-Enein, H.Y. (2006). Radical scavenging activity and antioxidant capacity of bay leaf extracts. *Journal Iran Chemistry Society*, 3,258-266.
7) Farhan, H., Rammal, H., Hijazi, A., Hamad, H., Badran, B. (2012a). Phytochemical screening and extraction of polyphenol from stems and leaves of a Lebanese *Euphorbia macroclada schyzoceras* Boiss. *Annual of Biological Research*, 3, 149-156.
8) Farhan, H., Rammal, H., Hijazi, A., Badran, B. (2012b). Preliminary phytochemical screening and extraction of polyphenol from stems and leaves of a Lebanese plant *Malva parviflora* L. *International Journal of Current Pharmaceutical Research*, 4,55-59.
9) Farhan, H., Rammal, H., Hijazi, A., Hamad, H., Daher, A., Reda, M., Badran, B. (2012c). In vitro antioxidant activity of ethanolic and aqueous extracts from crude *Malva parviflora* L. grown in Lebanon. *Asian Journal of Pharmaceutical and Clinical Research* 5(3), 234-238.
10) Farhan, H., Rammal, H., Hijazi, A., Daher, A., Reda, M., Annan, H., Chokr, A., Badran, B. (2013). Chemical composition and antioxidant activity of a Lebanese plant *Euphorbia macroclada schyzoceras*. *Asian Pacific Journal of Tropical Biomedicine* 3(7), 542-548
11) Frankel, E. (1995). Nutritional benefits of flavonoids. International conference on food factors: Chemistry and cancer prevention, Hamamatsu, Japan abstracts, C6-2.
12) Gupta, A., Naraniwal, M., Kothari, V. (2012). Modern extraction methods for preparation of bioactive plant extracts. *International Journal of Applied and Natural Sciences* 1, 8-26.
13) Rammal, H., Bouayed, J., Desor, F., Younos, C., Soulimani, R. (2009). Notes ethnobotanique et phytopharmacologique sur *Eryngium creticum* L. *Phytothérapie* 7, 26–27.
14) Hsu, C.L., Chen, W., Weng, Y.M., Tseng, C.Y. (2003). Chemical composition, physical properties, and antioxidant activities of yam flours as affected by different drying methods. *Food Chemistry* 83, 85-92.
15) Koleva, I.I., Van Beek, T.A., Linssen, J.P.H., deGroot, A., Estatieva, L.N. (2002). Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. *Phytochemical analysis* 13, 8-11.
16) Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* 193(1), 265-75.
17) Mallela, V.S., Ilankumaran, V., Srinivasa Rao, N. (2004). Trends in Cardiac Pacemaker Batteries. *Indian Pacing Electrophysiological Journal* 4(4), 201–212.
18) Mendel, R.R. (2009). Cell biology of molybdenum. *BioFactors* 35 (5), 429–34.
19) Smit, E.J., Gorris, L.G.M. (1999), Natural anti-microbials for food preservation, In: M.S. Rah-man, Handbook of Food Preservation, (pp. 285–308) Marcel Dekker, New York.
20) Sovljanski, R., Obradovie, S., Kisgeci, J., Lazie, S., Macko, V. (1989). The heavy metals contents and quality of hop cones treated by pesticide during the vegetation. *Acta Horticulture* 249, 81-88.
21) Walsh, L.M. (1971). Instrumental methods of Analysis of soils and plant tissues. Soil Science Society of America, Inc. Madison Wisconsin, USA, p 26-30.
22) WHO. (2002). Traditional Medicine Strategy: 2002–2005, World Health Organisation, Geneva, Switzerland, 1-6.