Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading doi:

Phytochemical screening and antioxidant activity of Lebanese *Eryngium creticum* L.

Hussein Farhan[1], Fatima Malli[1], Hassan Rammal[1,2*], Akram Hijazi[1], Ali Bassal[2], Nawal Ajouz[2], Bassam Badran[1]

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

ARTICLE INFO

Article history:
Received 15 September 2012
Received in revised form 27 September 2012
Accepted 28 October 201
Available online 28 December 2012

Keywords:
Eryngium creticum
Antioxidant activity
Phytochemical screening
Vitamin C

ABSTRACT

Objective: To determine the phytochemical screening and quantification of total phenolics contents in fresh *Eryngium creticum* (*E. creticum*) leaves and stems extract and to evaluate its total antioxidant activity. **Methods:** Quantification of total phenolics contents in fresh *E. creticum* leaves and stems extract and evaluation of its total antioxidant activity, were done using the spectrophotometric analyses. **Results:** The consumption of 100 g of fresh *E. creticum* leaves and stems could provide antioxidants equivalent to (78.50±0.80) mg of vitamin C and (50.42±0.50) mg of vitamin C, respectively. **Conclusions:** From this study, it can be concluded that *E. creticum* can be interesting to prevent diseases directly linked to oxidative stress.

1. Introduction

Medicinal plants are a source for a wide variety of natural products among which the phenolic acids and flavonoids are very interesting for their antioxidant properties. In addition to their ability to act as efficient free radical scavengers, their natural origin is an advantage to consumer in contrast to synthetic antioxidants whose uses are being restricted due to their carcinogenicity[1-3].

Oxidative stress is mediated by reactive oxygen species (ROS) which are generated during the normal and aberrant cellular metabolism that utilizes molecular oxygen. The imbalance between production of ROS and the capacity of the normal detoxification systems in favor of the oxidants leads to oxidative stress, which itself leads to cellular damage caused by the interaction of ROS with cellular constituents. Oxidative stress is involved in many acute and chronic diseases including cancer, cardiovascular troubles and neurodegenerative diseases. The balance between antioxidation and oxidation is believed to be critical in maintaining a healthy biological system[4].

Eryngium creticum (*E. creticum*), a perennial plant which belongs to family Umbellifereae is commonly known as Field Eryngo. 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[5]. *E. creticum* also showed anti-inflammatory and anti-microbial activities[6]. It was also used in the treatment of liver diseases, poisoning, anemia and infertility[7]. This plant has shown an antioxidant property by inhibiting the lipid peroxidase in the liver of the rat[8].

Recently, many researchers have taken a great interest in medicinal plants for their phenolic concentrations and related total antioxidant potential. It is also reported that some medicinal plants contain a wide variety of natural antioxidants, such as phenolic acids, flavonoids and tannins, which possess more potent antioxidant activity than dietary plants[1-3.9]. Many investigations indicate that these compounds are of great value in preventing the onset and/or progression of many human diseases[10]. The health-promoting effect of antioxidants from plants is thought to arise from their protective effects by counteracting reactive oxygen species (ROS)[1-3]. The best way to give antioxidant nutrients to the human body is to eat generous servings of fruits and vegetables rich in antioxidants, such as polyphenols[1-3]. The protective effects of dietary phytochemicals against oxidative stress-related diseases

*Corresponding author: Dr Hassan Rammal, Lebanese University, Doctoral School of Science and Technology, Research Platform for Environmental Science (PRASE).
Tel: 0096170885686
E-mail: hasanrammal@hotmail.com
Foundation Project: This work is financially supported by the Research Platform for Environmental Science (PRASE), Lebanese University.

are due to their contribution to the maintenance of redox homeostasis in cells[11].

The purposes of this study were to determine for the first time, the phytochemical screening of the Lebanese *E. creticum* and to evaluate the antioxidant power of the aqueous extract of the fresh leaves and stems of this plant. Spectrophotometric analyses were employed for the determination of total phenolics concentrations. Also, the total antioxidant activity value was quantified by the vitamin C equivalent antioxidant capacity (VCEAC) test. On the other hand, the antioxidant power of this plant was also determined using hydrogen peroxide radical.

2. Materials and methods

2.1. Plant collection and extraction

Fresh leaves and stems of *E. creticum* were gathered from the south region of Lebanon. Grinded leaves and stems (100 g) were macerated in 300 mL of pure water for 12 h at room temperature, and then for 12 h at 37 ℃. The filtrate was lyophilised, so 5 g extract was obtained[12].

2.2. Phytochemical screening

The different steps of the phytochemical screening were made according to Muanda *et al*[13].

2.3. Determination of the amount of vitamin C

A volume of 2 mL of standard solution of ascorbic acid (1 mg/1 mL) contains 2 mg of ascorbic acid. V2 is the volume of the stain necessary to the titration of the standard solution of vitamin C containing 2 mg of ascorbic acid. So, 1 mL of the solution of stain will be necessary to the titration of a solution containing X g of ascorbic acid.

$X(g) = (2\ mg \times 1\ mL) / V_2$

Y is the mass of ascorbic acid in the extract.

$Y(g) = (X_g \times V_3) / 1\ mL$

2.4. Determination of chlorophyll

The determination of the amount of total chlorophyll, chloropyll 'a' and 'b' was realized according to the method of AOAC (1990)[14]. Briefly, 5 g of the fresh leaves of *E. creticum* were grinded with 0.1 g of $CaCO_3$ and 25 mL of acetone. The obtained solutions were then filtered on Whatman paper No. 4 and the filtrate was picked up.

The leaves were grinded again with 40 mL acetone and 10 mL ether. The obtained solution was filtered and the filtrate was picked up.

The same operation was repeated with 10 mL ethyl ether until the disappearance of the green color of the leaves.

All the filtrates were then mixed together. V is the volume of the mixed filtrate. Different dilutions were done in order to make the color of the extracts more light. We carry out the same steps with 5 g of fresh stems. The control was prepared by mixing acetone and diethyl ether.The spectrophotometer (Analytric Jenna, specord 50) was used to measure the optical density at 660 and 642.5 nm. The content in chlorophyll (in mg/L) was determined from the followed equations: Total chloropyll: $(7.12 \times A_{660}) + (160.8 \times A_{642.5})$; Chloropyll a: $(9.93 \times A_{660}) - (0.777 \times A_{642.5})$; Chloropyll b: $(17.6 \times A_{642.5}) - (2.81 \times A_{660})$.

2.5. Determination of total phenolics

Total phenolics content was evaluated using the spectrophotometric analysis (Cary 50 Scan UV-Visible apparatus) with Ciocalteu's phenol reagent[2,3]. Briefly, an aliquot (1 mL) of appropriately diluted extract or standard solutions of caffeic acid (20, 40, 60, 80 and 100 mg/L) was added to a 25 mL volumetric flask containing 9 mL of ddH2O. A reagent blank using ddH_2O was prepared. One mililiter of Folin & Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 10 mL of 7 % Na_2CO_3 solution was added with mixing. The solution was then immediately diluted to volume (25 mL) with ddH_2O and mixed thoroughly. After incubation for 90 min at 23 ℃, the absorbance versus prepared blank was read at 765 nm. Total phenolics content in *E. creticum* leaves and stems was expressed as mg caffeic acid equivalent (CAE)/100 g fresh sample. Sample was analyzed in three replications.

2.6. Determination of total antioxidant activity using ABTS radical scavenging capacity assay

ABTS radicals were used to evaluate the antioxidant capacity of *E. creticum* fruit[1]. In brief, 1 mM AAPH, a radical initiator, was mixed with 2.5 mM ABTS in phosphate-buffered saline (PBS, pH 7.4). The mixed solution was heated in a water bath at 68 ℃ for 13 min. The resulting blue-green ABTS radical solution was adjusted to the absorbance of (0.650±0.020) at 734 nm with additional PBS. A volume of 20 μL of sample was added to 980 μL of the ABTS radical solution. The mixture was incubated at 37 ℃ water bath under restricted light for 10 min. A control consisted of 20 μL 50 % methanol and 980 μL of ABTS radical solution. The decrease of absorbance at 734 nm was measured 10 min later. Total antioxidant capacity of MC fruit, as determined by scavenging blue-green ABTS radicals, was expressed on a fresh weight basis as mg/100 g vitamin C equivalent (VCEAC). Sample was analyzed in three replications.

2.7. Scavenging activity of hydrogen peroxide (H_2O_2) radical

The hydrogen peroxide scavenging of the aqueous extract of *E. creticum* was determined according to the method of Ruch *et al*[23]. 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 stems and leaves extract of both plants (5, 10, 15, 20 and 25 mg /mL) in distilled water were added to a H2O2 solution (0.6 mL, 40 mM) and the absorbance of H_2O_2 at 230 nm was determined after 10 min against two blank solutions, the first contains PBS without H_2O_2 and the second contains PBS with H_2O_2.

The percentage scavenging of hydrogen peroxide was calculated using the following equation:

$\%\ \text{Scavenged}\ [H_2O_2] = [(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \times 100.$

3. Results

The chemical compositions of *E. creticum* were reported in Table 1.

Table 1.
The phytochemical screening of the fresh leaves and stems of E. creticum.

Test done	Leaves	Stems
Alkaloide	–	–
Dragendorff	++++	++
Mayer	++++	++
Tanin	+++	++
Catechic tanin	+++	+
Gallic tanin	+++	+
Flavonoid (anthocyane)	–	–
Saponin	++	–
Narcotic (tetrahydrocanabiol)	–	–
Flavonoids (cyanidine reaction)	–	–
Reducing agent	–	–
Mucilage	–	–
Coumarin	++	+

–: Negative result; ++++: Strongly positive; +++: Positive results; ++: Moderately positive.

3.1. Determination of vitamin C

After titration, the different obtained volumes were as follow:

$V_{1\ leaves}$ = 8 mL corresponds to V_3 = 1.8 mL

V_{1stems} = 14 mL corresponds to V_3 = 0.7 mL

V2= 29.5 mL

X (g) = (2 mg×1 mL) /V_2 = (2 mg×1 mL) /29.5 mL = 0.067 g

For the leaves: Y (g) = (X (g)×V_3)/1 mL = (0.067 g×1.8 mL) /1 mL= 0.12 g.

For the stems: Y (g) = (X (g)×V_3) /1 mL = (0.067 g×0.7 mL) /1 mL = 0.05 g.

3.2. Determination of chlorophyll

In leaves:

A_{660} =0.197 and $A_{642.5}$ = 0.074

Total chloropyll: (7.12×A_{660}) + (16.8×$A_{642.5}$) = (7.12×0.197×10) + (16.8×0.074×10) = 26.4584

Chloropyll a: (9.93×A_{660}) − (0.777×$A_{642.5}$) = (9.93×0.197) − (0.777×0.074) = 1.906592

Chloropyll b: (17.6×$A_{642.5}$) − (2.81×A_{660}) = (17.6×0.074) − (2.81×0.197) = 0.74883

In stems:

A_{660} = 0.076 and $A_{642.5}$ = 0.039

Total chloropyll: (7.12×A_{660}) + (16.8×$A_{642.5}$) = (7.12×0.076×10) + (16.8×0.039×10) = 11.9632

Chloropyll a: (9.93×A_{660}) − (0.777×$A_{642.5}$) = (9.93×0.076) − (0.777×0.039) = 0.724377

Chloropyll b: (17.6×$A_{642.5}$) − (2.81×A_{660}) = (17.6×0.039) − (2.81×0.076) = 0.47284

3.3. Determination of total phenolics

Figure 1 showed that the concentrations of total phenolics in fresh leaves and stems of *E. creticum* were 16.7 mg CAE and 9 mg CAE per 100 g fresh weight, respectively.

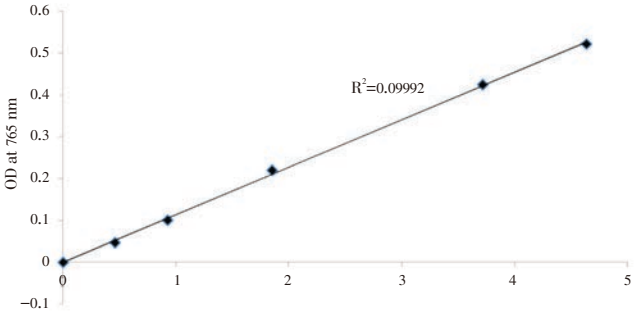

Figure 1. Concentration–response curve of caffeic acid.
The data were displayed with mean ± SD of three replications.

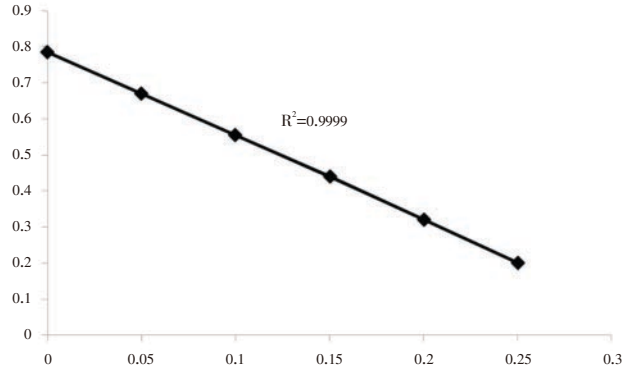

Figure 2. Concentration–response curve for reduction of ABTS radical by vitamin C.
The data were displayed with mean ± SD of three replications. Total antioxidant capacity estimated by VCEAC assay was expressed as vitamin C equivalent (VCE) per 100 g fresh weight.

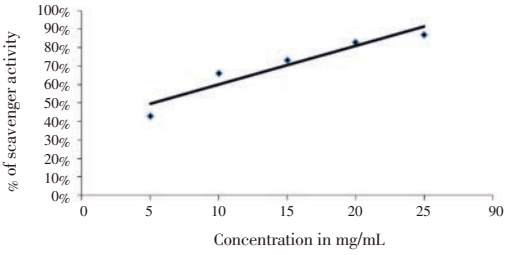

Figure 3. Hydrogen peroxide scavenging activity of the stems of *E. creticum*.
The data were displayed with mean ± SD of three replications.

Table 2.
Hydrogen peroxide (H_2O_2) scavenging activity of aqueous extract of the leaves and stems of *E. creticum* (mean±SD).

Concentrations (mg/mL)	Stems		Leaves	
	Absorbance	Inhibition (%)	Absorbance	Inhibition (%)
5	0.145 ± 0.028	43	0.240 ± 0.004	6
10	0.085 ± 0.004	66	0.207 ± 0.005	19
15	0.067 ± 0.009	73	0.187 ± 0.009	26
20	0.042 ± 0.006	83	0.053 ± 0.003	79
25	0.031 ± 0.004	87	0.008 ± 0.006	96

3.4. Antioxidant activity

Figure 2 showed the total antioxidant capacity, expressed as vitamin C equivalent antioxidant capacity (VCEAC), of fresh leaves and stems was (79.00 ± 0.80 mg) and (50.42 ± 0.50 VCE/100 g) fresh weights, respectively.

Also, Table 2, Figure 3 and Figure 4 showed that the free radical scavenging activity increased with increasing concentration of the extract. The obtained results showed that 25 mg/mL of the leaves of *E. creticum* induced 96% of inhibition of the H_2O_2.

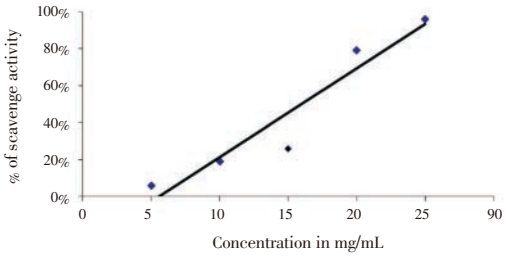

Figure 4. Hydrogen peroxide scavenging activity of the leaves of *E. creticum*.
The data were displayed with mean ± SD of three replications.

4. Discussion

The spectrophotometric analyses show that 100 g of fresh stems and leaves of *E. creticum* contain polyphenolics ranged from 9 mg to 16.7 mg of caffeic acid, respectively.

To evaluate antioxidant activities VCEAC test developed by Kim *et al*[16] was employed. This test is a good method for measuring the antioxidant activity of extracts or individual chemical compounds. The used parts of of *E. creticum* display scavenging activities for ABTS radical. We found that the total antioxidant activities varied greatly among these parts, since they ranged from (50.42 ± 0.50 mg) to (79.00 ± 0.80 mg) VCE/100 g fresh weights, respectively. Antioxidants are substances that delay the oxidation process, inhibiting the polymerization chain initiated by free radicals and other subsequent oxidizing reactions. Phenolic constituents, such as flavonoids, phenolic acids and tannins are well known for their high antioxidant activity[1-3,9]. The majority antioxidant capacity of plants is not only represented by vitamin C, vitamin E or β-carotene, but is also due to other compounds such as polyphenols which have a strong antioxidant potential[29]. Our findings implicate that dietary polyphenolics from *E. creticum* may supply substantial antioxidants, which may provide health-promoting advantages to the consumer. In the light of the obtained results, we can say that *E. creticum* used as salad in Lebanon displays scavenging activity for ROS and is potentially a source of natural antioxidants. This plant could be useful as therapeutic agents in the preventing and slowing the progress of aging, age-associated oxidative stresses-related degenerative diseases. In conclusion, this study highlights for the first time the antioxidant power of *E. creticum*, a potential which could be interesting to prevent diseases directly linked to oxidative stress.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

Authors wish to express their sincere gratitude to the Department of Biology for providing infrastructure facility. This research was funded by Research Platform for Environmental Science (PRASE), Lebanese University.

References

[1] Rammal H, Bouayed J, Hijazi A, Ezzedine M, Soulimani R. Scavenger capacity of Momordica charantia for reactive oxygen species. *J Nat Prod* 2012; **5**: 54-59.
[2] Farhan H, Rammal H, Hijazi A, Hamad H, Badran B. Phytochemical screening and extraction of polyphenol from stems and leaves of a Lebanese Euphorbia macrolada schyzoceras Boiss. *Ann Biol Res* 2012; **3**: 149-156.
[3] Farhan H, Rammal H, Hijazi A, Badran B. Preliminary phytochemial screening and extraction of polyphenol from stems and leaves of a Lebanese plant Malva parviflora L.. I*nt J Curr Pharm Res* 2012; **4**: 55-59.
[4] Hong H, Liu GQ. Protection against hydrogen peroxide-induced cytotoxicity in PC12 cells by scutellarin. *Life Sci* 2004; **74**: 2959-2973.
[5] Abu-Rabia A. Herbs as a food and medicine source in Israel. *Asia Pac J Cancer Prev* 2005; **6**: 101-107.
[6] Ali-Shtayeh MS, Yaghmour MR, Faidi YR, Salem K, Al-Nuri MA. Antimicrobial activity of 20 plants used in folkloric medicine in the Palestinian area. *J Ethnopharmacol* 1998; **6**: 265-271.
[7] Said O, Khalil K, Fulder S, Azaizeh H. Ethnopharmacological survey of medicinal herbs in Israel, the Golan Heights and the West Bank region. *J Ethnopharmacol* 2002; **83**: 251-265.
[8] Ljubuncic P, Azaizeh H, Portnaya I, Cogan U, Said O, Saleh K, et al. Antioxidant activity and cytotoxicity of eight plants used in traditional Arab medicine in Israel. *J Ethnopharmacol* 2005; **99**: 43-47.
[9] Bouayed J, Piri K, Rammal H, Dicko A, Desor F, Younos C, et al. Comparative evaluation of the antioxidant potential of some Iranian medicinal plants. *Food Chem* 2007; **104**: 364-368.
[10] Halliwell B, Gutteridge JMC, Cross CE. Free radicals, antioxidants, and human disease: where are we now? *J Lab Clin Med* 1992; **119**: 598-619.
[11] Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell B* 2007; **39**: 44-84.
[12] Rammal H, Bouayed J, Desor F, Younos C, Soulimani R. Validation et contribution à l'étude de l'effet antihyperglycémique d'une plante médicinale, le Momordica charantia L. Phytothérapie 2009; **7**: 191-196.
[13] Muanda F. Identification of polyphenols, evaluation of their antioxidant activity and study of their biological properties (thesis). France: University of Metz; 2010, p. 1-239.
[14] AOAC. Official methods of analysis of the association of official analytical chemists. 15th ed. Washington, DC: AOAC; 1990.
[15] Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis* 1989; **10**: 1003-1008.
[16] Kim DO, Jeong SW, Lee CH. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chem* 2003; **81**: 321-326.