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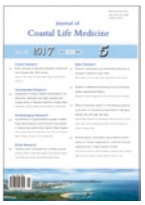

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Phenolic composition and antioxidant properties of *Eryngium maritimum* (sea holly)

Ilhem Rjeibi[1*], Anouar Ben Saad[1], Sana Ncib[2], Sami Souid[1]

[1]*Research Unit of Macromolecular Biochemistry and Genetic, Faculty of Sciences of Gafsa, Gafsa 2112, Tunisia*

[2]*Common Services Unit for Research, Faculty of Sciences of Gafsa, Gafsa 2112, Tunisia*

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ABSTRACT

Objective: To examine the antioxidant potential of Tunisian *Eryngium maritimum* (*E. maritimum*) leaf, root and stems extracts, as well as their phenolic compositions.
Methods: The antioxidant activity of different extracts was assessed using DPPH free radical and hydrogen peroxide scavenging assays. Phenolic profiles were determined by means of liquid chromatography (HPLC-DAD).
Results: All plant parts were a rich source of phenolics. Polyphenols and flavonoids were present in leaf extracts. *E. maritimum* leaf extracts displayed the strongest H_2O_2 scavenging activity (IC_{50} = 76.83 μg/mL) and the highest DPPH scavenging activity value (IC_{50} = 47.87 μg/mL) compared to other extracts. Good relationships were observed between antioxidant activities and the total phenolic and flavonoid contents. Nine bioactive compounds were detected in *E. maritimum* extracts.
Conclusions: Our results provided evidence that *E. maritimum* could be an interesting source of natural antioxidant that can be used to treat divers diseases.

1. Introduction

The genus *Eryngium* (Apiaceae) contains over 230 species widely distributed throughout the world[1]. Several members of this genus were consumed to prevent several health problems. In Asia and Africa, leaf of *Eryngium foetidum* was cultivated as vegetable[2]. In Turkish folk medicine, herbal infusions of *Eryngium bornmuelleri* (*E. bornmuelleri*) were traditionally used to treat and prevent several cancer types[3]. However, *Eryngium campestre* was used as aphrodisiac, antitussive, and appetizer. Roots of *Eryngium aquaticum* were used in folk medicine as anti-diuretic and antidote. *Eryngium maritimum* (*E. maritimum*) (sea holly) was reported to be used as diaphoretic, antihelminthic, and diuretic[4]. In addition, many researchers have studied biological properties of different species of this genus. According to Dalar *et al*.[5], the acetone and ethanol extract from *E. bornmuelleri* leaves showed antioxidant and enzyme inhibitory properties. Küpeli *et al*.[6] have also demonstrated that the aqueous and ethanol extract from the roots of eight *Eryngium* species possess anti-inflammatory and antinociceptive properties. Recently, Landoulsi *et al*.[7] reported that the essential oil extracted from leaves and roots of *Eryngium barrelieri* and *Eryngium glomeratum* possessed antimicrobial activity. Numerous studies on phytochemical characterization of various species had been carried out. Triterpenoids, coumarins, acetylenes, essential oil, flavonoids, and steroids were identified as the main constituents[8,7].

The objectives of this study were to assess the antioxidant activity of *E. maritimum* and to characterize the main phenolic compounds present in this plant.

2. Materials and methods

2.1. Plant materials

The leaves, roots, and stems of *E. maritimum* were sampled from North-Western Tunisia (36°57' 18" N latitude, 8°45'18" E longitude), in May 2015 and deposited at the herbarium in the Faculty of Sciences Gafsa, Tunisia.

*Corresponding author: Ilhem Rjeibi, Research Unit of Macromolecular Biochemistry and Genetic, Faculty of Sciences of Gafsa, Gafsa 2112, Tunisia.
Tel: +21620480884
E-mail: rjeibii@yahoo.fr
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2.2. Preparation of extracts

Ten grams of the powdered herb from *E. maritimum* were infused into 200 mL of hot water for 30 min, centrifuged and lyophilized to obtain aqueous infusions from leaf (LEM), stems (SEM) and root extracts (REM), respectively.

2.3. Chemical properties

2.3.1. Determination of total phenolics

The amount of total phenolics (TPC) in leaves, roots, and stems was assayed using the protocol of Dewanto *et al.*[9]. Briefly, 125 µL of samples were mixed with 125 µL of Folin-Ciocalteu reagent (10%). After 1 min of incubation, 1 500 µL of sodium carbonate solution (7%) was added to the mixture and incubated in the dark for 90 min. The absorbance of the solution was measured at 760 nm in a UV-Vis spectrophotometer (Shimadzu, 1240 model, Tokyo, Japan). The analysis was performed in triplicate and TPC was expressed as gallic acid equivalent in milligrams per gram of extract.

2.3.2. Determination of total flavonoids

The amount of total flavonoids (TFC) in different plant parts was assayed using the protocol of Dewanto *et al.*[9]. Briefly, 1 000 µL of samples were mixed with 750 µL of sodium nitrite solution (5%). After 5 min, 0.15 mL of 10% aluminium chloride solution was added and the mixture was allowed to stand for a further 5 min, and then 0.5 mL of 1 mol/L sodium hydroxide (NaOH) was added to the solution. The absorbance was measured at 510 nm. TFC was expressed as milligrams of quercetin equivalent per gram of extract.

2.4. Antioxidant properties

2.4.1. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The radical scavenging capacity was monitored using the stable free radical DPPH following the method described by Tlili *et al.*[10]. Adequate solutions of each sample were created to obtain a final volume of 1 mL. Then, they were mixed with a freshly prepared DPPH solution (0.1 mmol/L). The tubes were shaken and incubated at room temperature for 30 min in the dark. The absorbance was measured at 515 nm.

2.4.2. Hydrogen peroxide scavenging assay

The H_2O_2 scavenging activity of different plant parts was determined according to the method reported by Liu *et al.*[11] with some modifications. 500 µL of sample dilution was mixed with 1 200 µL of phosphate buffer (0.1 mol/L, pH 7.4) and 300 µL of H_2O_2 solution (40 mmol/L). The mixture was shaken vigorously and incubated at room temperature for 10 min. The absorbance was determined at 230 nm.

H_2O_2 scavenging activity (%) = $[(A_{control} - A_{sample})/A_{control}] \times 100$

2.5. High-performance liquid chromatography (HPLC) analysis of bioactive compounds

The analyze of phenolic compounds were performed using a Varian ProStar HPLC System (Varian 330/Vis Detector and Varian 230 SDM). In the analyses, we used reverse phase chromatography equipped with C18 column (4.6 × 250 -mm). The mobile phase consisted of water containing acetic acid (solvent A) and methanol (solvent B). The gradient was composed of 0% (B) for 2 min, 50% (B) until 30 min, and 80% (B) for 5 min. The samples were utilized in the concentration of 1 mg/mL. The flow rate was 0.6 mL/min and the volume injected was 30 mL. The mobile phase and samples were subjected to filtration through 0.45 mm membrane filter (Millipore) and then degassed in ultrasonic before use. The detected compounds were identified by comparing their retention time with those of injected authentic standards and use of DAD spectra (200–600 nm). Analyses were performed in triplicate.

2.6. Statistical analysis

A statistical approach was designed and the experimental data were evaluated using Statistica version 10, software, with $P < 0.05$ as threshold for statistical significance. All tests were performed in triplicate and the results are expressed as mean ± SD.

3. Results

The extraction yields of the aqueous infusions from leaf, stems, and root of *E. maritimum* are shown in Table 1. The percentage yield was in the order of 19.8%, 4.14% and 1.22% for leaves, stems, and roots, respectively. The total phenolic and flavonoid contents of *E. maritimum* extracts are illustrated in Figure 1. Significant differences were observed depending in plant parts. The amounts of total phenolics varied from 8.09 to 1.69 mg GAE/g dry weight with a decreasing order: LEM > REM > SEM. The highest content of flavonoids was observed in leaves (1.03 mg CE/g dry weight), while the lowest content was found in stems (0.23 mg QE/g dry weight). The results of the *in vitro* antioxidant activity of *E. maritimum* are summarized in Table 1. Leaf, stems and root extracts indicated appreciable activities against DPPH and H_2O_2 scavenging radicals. The antioxidant activity determined using DPPH showed that the leaf extracts significantly ($P < 0.05$) exhibited the highest radical scavenging activity (IC_{50} = 47.87 µg/mL) followed by the root extracts (IC_{50} = 90.40 µg/mL) and stem extracts (IC_{50} = 117.29 µg/mL).

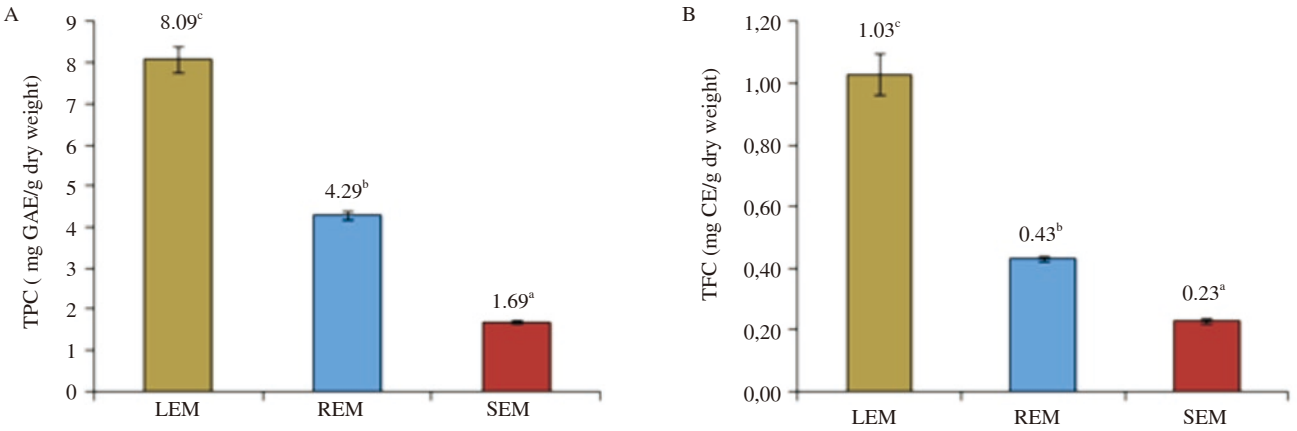

Figure 1. Total phenolic content (A) and total flavonoid content (B) of leaf extracts (LEM), stem extracts (SEM) and root extracts (REM) from *E. maritimum*. Means with different letters were significantly different at the level of $P < 0.05$. Each value is expressed as the mean ± SD of triplicate measurements.

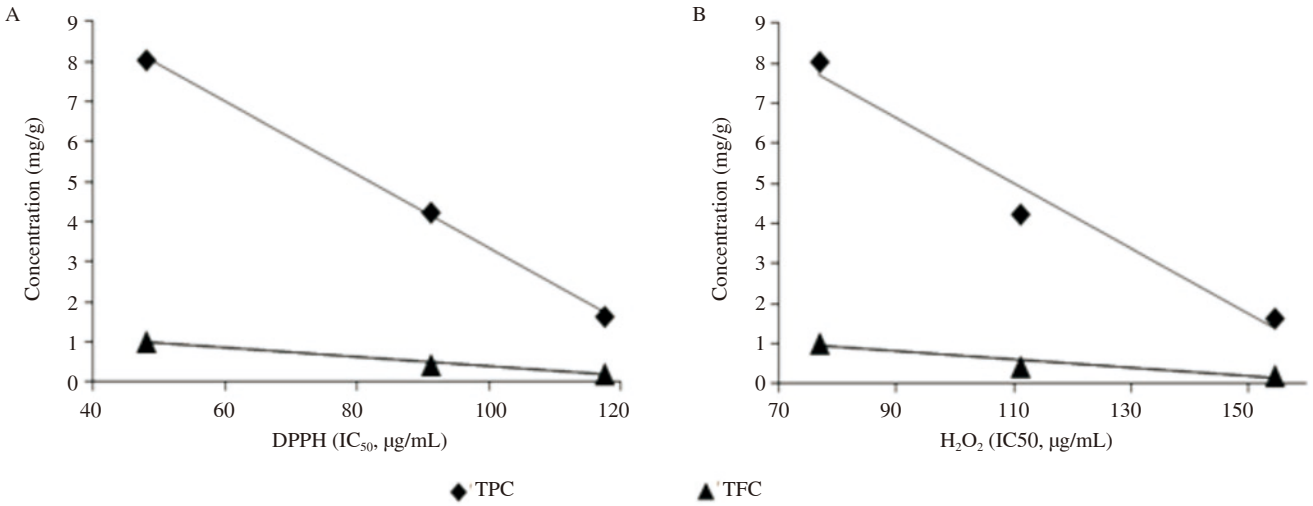

Figure 2. Correlation between polyphenolic contents and antioxidant activities of leaf extract from *E. maritimum*.
A: DPPH free radical-scavenging activity; B: Hydrogen peroxide scavenging activity.

Table 1
The yields and the antioxidant activities of *E. maritimum* extracts.

Extracts	Yields (%)	DPPH scavenging activity (IC_{50}, µg/mL)	H_2O_2 scavenging activity (IC_{50}, µg/mL)
Leaves extract	19.8 ± 1.21[c]	47.87 ± 0.51[a]	76.83 ± 0.58[a]
Stems extract	4.14 ± 0.23[b]	117.29 ± 1.23[c]	154.23 ± 2.14[c]
Roots extract	1.22 ± 0.65[a]	90.40 ± 0.87[b]	111.06 ± 2.01[b]
Vitamin C	-	19.12 ± 0.12	24.85 ± 0.15

Values are mean ± SD, $n = 3$. Different letters for the same column indicate significant differences at $P < 0.05$.

A strong correlation was found between DPPH scavenging ability and total polyphenolics content ($r^2 = 0.999$) as well as the total flavonoids content ($r^2 = 0.989$) (Figure 2). Once again, the leaf extracts showed the highest H_2O_2 scavenging activity ($IC_{50} = 76.83$ µg/mL) and good relationships were observed with TCP ($r^2 = 0.984$) and TFC ($r^2 = 0.940$). The results of the phenolic composition by RP-HPLC revealed that *E. maritimum* extracts is composed of six phenolic acids and three flavonoids (Table 2). Accordingly, *E. maritimum* was characterized by the predominance of caffeic acid (12.54%–2.58%). However, rutin (0.95%) was only detected in stem extracts. Other phenolic compounds were found in some extracts but absent in others.

Table 2
Main compounds identified in *E. maritimum* extracts by HPLC-DAD.

Compounds	Leaf extracts (%)	Stem extracts (%)	Root extracts (%)
Gallic acid	3.69 ± 0.78	3.02 ± 0.00	2.15 ± 0.03
Chlorogenic acid	4.75 ± 0.51	2.14 ± 0.12	1.03 ± 0.24
Catechin	0.74 ± 0.01	0.88 ± 0.01	0.55 ± 0.01
Vanillic acid	5.32 ± 0.21	ND	ND
Caffeic acid	12.54 ± 1.02	8.51 ± 1.54	2.58 ± 0.77
Rutin	ND	0.95 ± 0.12	ND
Quercetin	5.62 ± 0.37	ND	1.32 ± 0.12
Luteolin	1.55 ± 0.01	ND	0.65 ± 0.00
Cinnamic acid	3.78 ± 0.05	1.54 ± 0.55	3.66 ± 1.24

Results were given as means ± SD. ND: Not determined.

4. Discussion

Phenolic compounds derived from leaf, root, and stem play a very important role in the biological activities of medicinal plants such as anti-inflammatory, anti-tumor, antioxidant and anti-microbial effects[12]. The total phenolic and flavonoid contents, as well as the antioxidant property from different part of *E. maritimum* were investigated in the

present work. Phytochemical analysis of sea holly extracts revealed that the amount of phenolic compounds was considerable. The leaves had higher total phenolic and flavonoid contents than roots and stems. This may be explained by the influence of developmental stage of the plant on the distribution of phytoconstituents. These observations are consistent with the findings of Mohd-Esa *et al.*[13].

The antioxidant properties of *E. maritimum* evaluated using DPPH free radical scavenging activity and H_2O_2 scavenging assays showed that the *in vitro* activity is dependent on the different plant parts. Thus, leaf extract showed the highest antioxidant activity as compared to other extracts. This is in agreement with the results of Ebrahimzadeh *et al.*[14] who revealed a good antioxidant proprieties of leaf extract from *Erythronium caucasicum* as compared to the flowering stages. Their important antioxidant capacities were essentially linked to the high amount of TPC and TFC. In fact, a good relationship was observed between the phenolic and flavonoid contents and the antioxidant activity of *E. maritimum* extracts. This finding is in agreement with results of other authors, who demonstrated such linear correlation[5]. Moreover, the difference in the radical scavenging activity of *E. maritimum* extracts could be associated with the nature of their phenolic compounds. Accordingly, six phenolic acids (gallic acid, catechin, chlorogenic acid, vanillic acid, caffeic acid and cinnamic acid) and three flavonoids (rutin, quercetin and luteolin). Many works on the therapeutic potentials of these compounds have been reported. For instance, chlorogenic and caffeic acids were reported as potent *in vivo* and *in vitro* antioxidant[15]. In addition, Dalar *et al.*[5] have suggested that rutin, quercetin, luteolin and caffeic acid obtained from *E. bornmuelleri* leaf extract may be responsible for their antioxidant properties.

Thus the result revealed that the infusion obtained from the leaf extracts of *E. maritimum* is a recommended natural antioxidant essential for the treatment of disorders caused by free radicals. However, future studies are necessary to identify which of individual phenolic compounds present in *E. maritimum* leaves are implicated in the antioxidant activity.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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