Current Research Journal of Biological Sciences 4(4): 437-443, 2012
ISSN: 2041-0778

Submitted: February 22, 2012 Accepted: March 12, 2012 Published: July 10, 2012

Eryngium maritimum: A Rich Medicinal Plant of Polyphenols and Flavonoids Compounds with Antioxidant, Antibacterial and Antifungal Activities

[1]Wahiba Kholkhal, [2]Faiza Ilias, [1]Chahrazed Bekhechi and [1]Fawzia Atik Bekkara
[1]Laboratory of Natural Products, Department of Molecular and Cellular Biology, Faculty of Science, University Abou Bekr Belkaid, Nouveau Pôle Rocade II Tlemcen 13000, Algérie
[2]Laboratory of Ecology and Management of Ecosystems, Department of Biology and Environment, Faculty of Science, University Abou Bekr Belkaid, Nouveau Pôle Rocade II Tlemcen 13000, Algérie.

Abstract: In this study *Eryngium maritimum* roots were collected from plants grown at Tlemcen in west Algeria. They were powdered and extracted why to assess, the one hand, total phenolic and flavonoid content, on the other hand, antioxidant, antibacterial and antifungal activities of (methanol extract, acetone extract, ethyl acetate fraction and butanol fraction). The results showed that the amount of total phenolic and flavonoid content was higher in acetone extract (55.80±2.75 mg PE/g DW, 1.505±0.013 mg RE/g DW) than methanol extract (46.72±4.69 mg PE/g DW, 1.138±0.016 mg RE/g DW). Higher Free Radical Scavenging and iron reducing power of butanol extract was observed (0.0104±0.0004 and 0.1139±0.0112 mg/mL). Results of antibacterial activity showed that *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus* were inhibited by all the extract. Thus, only methanol and butanol extract was active against *Pseudomonas aeruginosa*. Results showed that ethyl acetate extract revealed the strongest activity against all the test fungi specially, toward *Aspergillus flavus* (10 mm at 50 mg/mL).

Keywords: Antibacterial activity, antifungal activity, antioxidant activity, *Eryngium maritimum*, flavonoids, polyphenols

INTRODUCTION

It is noteworthy that drugs derived directly or indirectly from natural compounds play even today a major part in drug discovery and development in the areas of cancer and infectious diseases (Cragg *et al*., 1997; Newman *et al*., 2003).

Whereas, it has been established that oxidative stress is among the major causative factors in the induction of many chronic and degenerative diseases including: atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others (Diaz *et al*., 1997; Heinecke, 2003). The most effective way to eliminate free radicals which cause the oxidative stress is with the help of antioxidants. Antioxidants, both exogenous or endogenous, whether synthetic or natural, can be effective in preventing free radicals formation by scavenging them or promoting their decomposition and suppressing such disorders (Maxwell, 1995; Halliwell, 2000; Cesquini *et al*., 2003). There is our days, an increasing interest in the measurement and use of plant antioxidants for scientific research (Ksouri *et al*., 2009). This is mainly due to their strong biological activity, exceeding those of many synthetic antioxidants which have possible activity as promoters of carcinogenesis (Suhaj, 2006).

The most prominent family of antioxidants from plants is represented by phenolic compounds. This fact is well reflected through the large number of plants containing phenolic compounds as dominant active principles (Antal, 2010). Polyphenols constitute one of the most numerous and widely-distributed groups of substances in the Plant Kingdom, with more than 8000 phenolic structures currently known (Urquiaga and Leighton, 2000). Among phenolic compounds, flavonoids represent the most common group (Antal, 2010).

On the other hand, because of the resistance of that pathogens build against antibiotics, there is a growing interest to use natural antibacterial products for food preservation, like extracts of herbs and spices (Smid and Gorris, 1999). The activity of plant extracts on bacteria and fungi has been studied by a very large number of researchers in different parts of the world (Vuuren and Naidoo, 2010; Bhengraj *et al*., 2008).

The genus *Eryngium*, belonging to the subfamily Saniculoidea of Apiaceae, was represented by 317 accepted taxa worldwide (Erdelmeier and Sticher, 1986). One of this species it known in Algerian folk-medicine:

Corresponding Author: Wahiba Kholkhal, Laboratory of Natural Products, Department of Molecular and Cellular Biology, Faculty of Science, University Abou Bekr Belkaid, Nouveau Pôle Rocade II Tlemcen 13000, Algérie

Eryngium maritimum (Lahiet el Maaza, Kounida). *Eryngium maritimum* or Sea Holly was an perennial evergreen plant with spiny and silvery leafs and small summer flowers. He grows on the Euro-Mediterranean coastlines, rather on the sandy grounds (Quezel and Santa, 1963; Andrew, 2001).

Infusion of aerial and roots parts of this species are used in folk remedies as antitussive, diuretic, appetizer, stimulant and aphrodisiac (Baytop, 1999). The roots part, also, causes menstruation, promotes flatulence, cystotonic, urethritis remedy, stone inhibitor and removes obstructions liver, kidney and gall-bladder (Andrew, 2001). In England, it froze the root of Sea Holly, which is consumed like candy, but also to prevent scurvy (Andrew, 2001). In Algeria, powdered root was used in soup in order to cause menstruation. Phytochemical investigations on the Sea Holly indicated the presence of Flavonoids, Saponins, Coumarins and Acids (Andrew, 2001).

In fact of numerous applications in traditional medicine of *Eryngium maritimum* roots, it tried to investigate his polyphenols and flavonoids content and evaluate the antibacterial, antifungal and antioxidant activities of methanol extract, acetone extract, ethyl acetate extract and butanol extract. In Algeria there is no study on this plant.

MATERIALS AND METHODS

Collection of plant materials: *Eryngium maritimum* roots was collected from plants grown in the region of Tlemcen in west Algeria on 2010, it was identified by "Laboratory of Ecology and Management of Ecosystems, University of Abou Bekr Belkaid, Tlemcen, Algeria" and the voucher specimens have been deposited at the Herbarium of "Natural Product Laboratory, University of Abou Bekr Belkaid, Tlemcen, Algeria".

Plant extraction: Fresh roots of *Eryngium maritimum* was air-dried, ground into a fine powder and stored at 4ºC until use. 5, 5 and 10 g of powdered roots were weighted into adequate glass beaker and 100 mL of Extract of aqueous acetone (70%), 100 mL of Extract of aqueous methanol (80%) and 200 mL of Extract aqueous methanol (80%) were added, respectively. The beakers were suspended in a water bath and homogenized with an (ULTRA TURRAX, IKA^R WERKE) at 13500 rpm for 30 min at 4ºC. The content of each beaker was filtered separately through filter paper. The residue was again treated with similar manner.

Determination of total phenolic and flavonoid content: They were determined using extract sample of aqueous acetone and aqueous methanol.

Determination of total phenolic content: The amount of total phenolic content was determined by Folin-Ciocalteu procedure (Singleton and Rossi, 1965). Aliquot (0.1 mL) of each sample extract was transferred into the test tubes and their volumes made up to 3 mL with distilled water. After addition of 0.5 mL Folin-Ciocalteu reagent and 2 mL of 20% aqueous sodium carbonate, tubes were vortexed and incubated at room temperature under dark condition. The absorbance was recorded after 1 h at 650 nm JEN WAY 6405 UV/Vis spectrophotometer. The total phenolic content was calculated as a Pyrocatechol equivalent (mg PE/g DW), from the calibration curve of Pyrocatechol standard solutions (range 1-15 mg/mL), giving an equation as:

Absorbance = 0.0132 Pyrocatechol (mg/mL) - 0.035 (R^2 = 0.997)

All tests were carried out in triplicate.

Determination of total flavonoid content: It was determined based on the formation of flavonoid-aluminium (Djeridane *et al*., 2006). 1 mL of each sample extract was mixed with 1 mL 2% aluminium chloride solution. After incubation for 15 min at room temperature, the absorbance at 430 nm was determined in JEN WAY 6405 UV/Vis spectrophotometer. The calibration curve was performed with Rutine (range 0.1.1 mg/mL), giving an equation as:

Absorbance = 2.302 Rutine (mg/mL) + 0.021 (R^2 = 0.992)

The results are expressed as Rutine equivalent (mg QE/g DW). Tests were carried out in triplicate.

Extraction of flavonoids: Sample extracts of aqueous methanol were evaporated to dry under reduced pressure at 45ºC. The dried weight obtained were measured and treated with 10 mL of hot distilled water in order to dissolve flavonoids. Then, they were extracted with ethyl acetate (3×10 mL). The remaining extract was continuously extracted with n butanol (3×10 mL). Ethyl acetate extracts and n butanol extracts were washed with dried Na_2SO_4 and evaporated to dryness under reduced pressure at 45ºC. The dried weight of each extract were measured and stored at 4ºC for further tests (Bekkara, 1999).

Antioxidant, antibacterial and antifungal activities: The antioxidant, antibacterial and antifungal activities of aqueous acetone extract, aqueous methanol extract, ethyl acetate fraction and butanol fraction of *Eryngium maritimum* roots were assessed.

Antioxidant activity:

Free radical scavenging activity: The procedure of (Brand-Williams, 1995) was adapted for evaluation of the free-radical scavenging capacity of sample extracts.

Briefly, dried extracts were dissolved in methanol why obtained different concentration. The assay mixture contained in total volume of 1 mL, 500 µ1 of the extract, 125 µL prepared DPPH (1 mM in methanol) and 375 µL of methanol. Ascorbic acid was used as a positive control. After 30 min incubation at 25°C, the decrease in absorbance was measured at λ = 517 nm in JEN WAY 6405 UV/Vis spectrophotometer against blank of each concentration (extract plus methanol). The capacity to scavenge the DPPH radical was calculated as follows:

Radical scavenging activity percent = $\left(\frac{A-B}{A}\right)\times 100$:

where, A is the absorbance of the negative control (DPPH plus methanol) and B is the absorbance of the sample (DPPH, methanol plus sample). The correlation between each concentration and its percentage of scavenging was plotted and the EC_{50} was calculated by interpolation. The activity was expressed as EC_{50}(the effective concentration of each extract that scavenges 50% of DPPH radicals).

Iron reducing power: The capacity of plant extracts to reduce Fe3+ was assessed by the method of (Oyaizu, 1986). Each dried extract were dissolved with methanol and different concentration (0.1, 0.25, 0.5, 0.75 and 1 mg/mL) were prepared. One milliliter of each one was mixed with 2.5 mL of sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide and the mixture was incubated at 50ºC for 20 min. After that, 2.5 mL of 10% trichloroacetic acid were added and the mixture was centrifuged at 3000 g for 10 min. The upper layer fraction (2.5 mL) was mixed with 2.5 mL of deionized water and 0.5 mL of 0.1% ferric chloride and thoroughly mixed. The absorbance was measured at 700 nm and ascorbic acid was used as a positive control. The EC_{50} value (mg/mL) is the extract concentration at which the absorbance was 0.5 for the reducing power and was obtained from the linear regression equation prepared from the concentrations of the extracts and the absorbance values. A higher absorbance indicates a higher reducing power.

Antibacterial activity: Growth inhibition activities for sample extracts against (*Staphylococcus aureus* ATCC 25923, *Enterococcus feacalis* ATCC 29212, *Bacillus cereus* ATCC 10876, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Listeria monocytogenes* ATCC 15313, *Klebsiella pneumonia* ATCC 700603, *Enterobacter cloacae* ATCC 13047, *Citrobacter freundii* ATCC 8090, *Proteus mirabilis* ATCC 35659 and *Salmonella typhemurium* ATCC 13311) were tested using disc diffusion method as described by (Berghe and Vlietinck, 1991). The suspension of bacteria of about 1.5 $\times 10^6$ CFU/mL. colony forming units per milliliter obtained following a 0.5 McFarland turbidity standard, which was standardized by adjusting the optical density to 0.1 at 600 nm (JEN WAY 6405UV/Vis spectro spectrophotometer) (Tereschuck *et al*., 1997). One milliliter of inoculums suspensions were used to inoculate by flooding the surface of Mueller-Hinton Agar plates. Excess liquid was air-dried under a sterile hood. Dried extract were dissolved in DMSO at the concentration 25, 30 and 50 mg/mL for aqueous methanol extract and aqueous acetone extract and 10, 15 and 20 mg/mL of ethyl acetate fraction, butanol fraction. After, sterilized discs (Whatman nº1, 6 mm diameter) were impregnated with 5 µL of each extract (equivalent to 125, 150 and 250 µg/disc for aqueous methanol extract and aqueous acetone extract, respectively and equivalent to 50, 75 and 100 µg/disc for ethyl acetate fraction and butanolic fraction, respectively) and placed on the agar surface. DMSO was used as negative control. The plates were left for 30 mn at room temperature to allow the diffusion of extract and then they were incubated at 37ºC for 24 h. Antibacterial activity was evaluated by measuring the diameter of the inhibition zone and presented in millimeter.

Antifungal activity: Determination of antifungal activity of sample extracts was accomplished by agar disk diffusion method (NCCLS, 1997). The dried extracts were dissolved in DMSO to a final concentration of 10, 15, 20, 30 and 50 mg/mL for ethyl acetate fraction, butanolic fraction, aqueous methanol extract and aqueous acetone extract. Antifungal tests were carried out using the disc diffusion method reported by Murray *et al*., (1995) and employing 100 µL of suspension containing 10^4 spore/mL of (*Cladosporium herbarum* MNHN 3369, *Alternaria alternata* MNHN 843390, *Aspergillus fumigatus* MNHN 566, *Aspergillus flavus* MNHN 994294) on the Potato Dextrose Agar. The disks (6 mm in diameter) impregnated with 5 µL of the extracts solution (equivalent to 50, 75, 100, 150 and 250 µg/disc) and DMSO (as negative control) were placed on the inoculated agar. The inoculated plates were incubated for 72 h at 30ºC. The diameters of inhibition zones were used as a measure of antifungal activity and each assay was repeated twice.

Statistical analysis: Assays were performed in triplicate for each sample. Results were expressed as mean values±Standard Deviation (SD). Correlation and regression analysis were carried out using the Origin Pro 8 SRO v8.0724 (B724).

RESULTS

Extract yield: Table 1 showed the extraction yielding obtained for each extraction from *Eryngium maritimum* roots. The same tendency was observed as in methanol extract (5.6±0.1%) as in acetone extract (5.6±0.4%). But, butanol extract yield was most important (3±0.2%) compared to ethyl acetate extract yield (0.3±0.02%).

Determination of total phenolic and flavonoid content: The amount of total phenolic was higher in acetone

Table 1: Yield of different extracts from *Eryngium maritimum* roots

Extract	Yield (%)
Methanol extract	5.6±0.1
Acetone extract	5.6±0.4
Ethyl acetate extract	0.3±0.02
Butanol extract	3±0.2

Table 2: Total phenolic and flavonoid of different extracts from *Eryngium maritimum* roots

Extract	Total phenolic (mg PE/g dw)	Total flavonoid (mg RE/g dw)
Methanol extract	46.72±4.69	1.138±0.016
Acetone extract	55.80±2.75	1.505±0.013

Table 3: Results of free radical scavenging activity and reducing power of different extract from *Eryngium maritimum* roots

Extract	IC_{50} (mg/mL) on DPPH	IC_{50} (mg/mL) on reducing power
Methanol extract	0.2350±0.0056	0.4719±0.0000
Acetone extract	0.0818±0.0048	0.3648±0.0099
Ethyl acetate extract	0.12±0.0062	0.3972±0.0022
Butanol extract	0.0104±0.0004	0.1139±0.0112
Ascorbic acid	0.008±0.0001	0.0203±0.0018

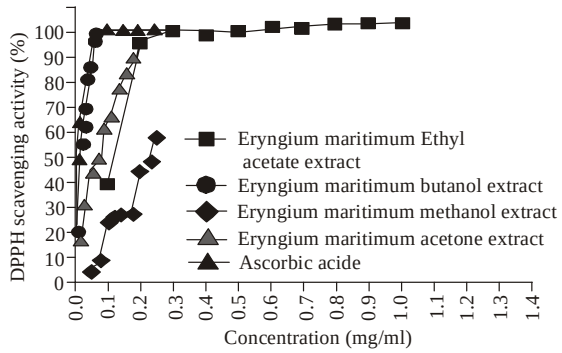

Fig. 1: DPPH radical scavenging activities of each extracts from *Eryngium maritimum* roots

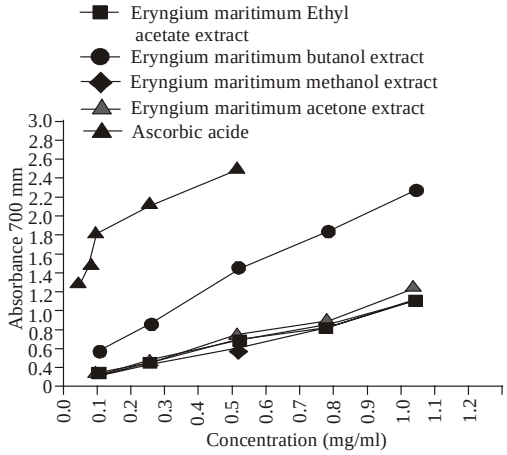

Fig. 2: Iron reducing power of each extracts from *Eryngium maritimum* roots

extract 55.80±2.75 mg PE/g DW than methanol extract 46.72±4.69 mg PE/g DW (Table 2). Flavonoid content was also higher in acetone extract 1.505±0.013 mg RE/g DW than methanol extract 1.138±0.016 mg RE/g DW.

Antioxidant, antibacterial and antifungal activities:

Antioxidant activity:

Free radical scavenging activity: The stable DPPH radical is widely used to evaluate the free radical scavenging activity in many plant extracts. The results of DPPH test (Table 3, Fig. 1) showed that butanol extract was the most active with an IC_{50} value of 0.0104±0.0004 mg/mL followed by acetone extract, ethyl acetate extract and methanol extract with IC50 value of 0.0818±0.0048, 0.12±0.0062 and 0.2350±0.0056 mg/mL. These plant extracts showed a slight lower radical scavenging activity compared to Ascorbic acid ($IC_{50,}$ 0.008±0.0001 mg/mL).

Table 4: Antibacterial activity of each extracts from *Eryngium maritimum* roots at different concentration

	Inhibition zone (mm) against										
Extract (mg/mL)	*S.a*	*K.p*	*L.m*	*En.c*	*E.f*	*E.c*	*C.f*	*P.a*	*P.m*	*B.c*	*S.t*
	ME										
25	7.5	-	7	6	6	7	6	6	7	7	6
30	8	-	8	6.16	7	7.18	6.16	6.16	7.18	7.18	6.5
50	8.5	6	9	6.5	7.5	7.5	6.5	6.5	7.5	7.66	7
	AC										
25	-	-	7	6	6.5	6.5	7	-	6	9	6
30	-	-	9	6.16	6.83	6.83	7.18	-	7	9.33	6.5
50	6	-	11	6.5	7	7	7.66	-	8	9.5	7
	EtA										
10	6	7	-	7	8	7	7.33	-	7	9	7
15	7	7.66	6	7.66	8.33	7.18	7.66	-	7.33	9.33	8
20	9	8	7	8	8.83	7.66	8	-	7.66	9.66	9
	Bu										
10	6.5	7	8.5	8	-	6	6.5	8	-	7.5	-
15	6.83	7.18	8.83	8.5	-	6.16	6.83	8.33	-	7	-
20	7	7.5	9	9	-	6.5	7	8.5	6	8.5	6

Extract: ME (Methanol extract), AC (Acetone extract), EtA (Ethyl Acetate extract), Bu (n-Butanol extract); Bacteria: *S.a* (*Staphylococcus aureus*), *K.p* (*Klebsiella pneumonia*), *L.m* (*Listeria monocytogenes*), *En.c* (*Enterobacter cloacae*), *E.f* (*Enterococcus feacalis*), *E.c* (*Escherichia coli*), *C.f* (*Citrobacter freundii*), *P.a* (*Pseudomonas aeruginosa*), *P.m* (*Proteus mirabilis*), *B.c* (*Bacillus cereus*) and *S.t* (*Salmonella typhemurium*)

Table 5: Antifungal activity of each extracts from *Eryngium maritimum* roots at different concentration

	Inhibition zone (mm) against			
Extract (mg/mL)	*A.a*	*Cl.h*	*A.fl*	*A.fu*
	ME			
20	-	7.18	-	6.83
30	-	8	-	8
50	-	9.33	-	9
	AC			
20	-	-	-	-
30	-	7.18	-	-
50	-	8	-	7
	EtA			
20	6.83	7	-	7.5
30	7.5	8	8.66	8
50	8.83	9	10	9.66
	Bu			
20	-	-	-	-
30	-	7	-	8.66
50	-	8	-	10

Extract: ME (Methanol extract), AC (Acetone extract), EtA (Ethyl Acetate extract), Bu (n-Butanol extract), *A.a* (*Alternaria alternata*), *Cl.h* (*Cladosporium herbarum*), *A.fl* (*Aspergillus flavus*) and *A.fu* (*Aspergillus fumigatus*)

Iron reducing power: Iron reducing power of a compound may serve as indicator of its potential antioxidant activity. As shown in Table 3 and Fig. 2 the reducing power of butanol extract, expressed as IC_{50}, was higher than other extract plant (0.1139±0.0112 mg/mL), followed by acetone extract, ethyl acetate extract and methanol extract with IC_{50} value of 0.3648±0.0099, 0.3972±0.0022 and 0.4719±0.0000 mg/mL. Ascorbic acid was a higher reducing activity (0.0203±0.0018 mg/mL).

Antibacterial activity: Table 4 showed the antibacterial activity of sample extracts measured by the agar diffusion method against selected pathogenic bacteria. Results indicated that all the extracts were a higher activity specially, toward *Bacillus cereus*.

Antifungal activity: The antifungal activity of the different sample extracts are presented in Table 5. Results showed that ethyl acetate extract revealed the strongest activity against all the test fungi specially, toward *Aspergillus flavus* (10 mm at 50 mg/mL). Then, methanol extract, acetone extract and butanol extract had a highest inhibitory activity against *Cladosporium herbarum* and *Aspergillus fumigatus*, but no inhibitory activity toward *Alternaria alternata* and *Aspergillus flavus*.

DISCUSSION

In this study, extract yield, total phenolic and flavonoid content, antioxidant and antimicrobial activities of *Eryngium maritimum* roots were determined.

By comparing our results of acetone and methanol extract yield with the bibliography, one found of roots ethanolic and aqueous extract of *Eryngium maritimum* in (Küpeli *et al*., 2006) is considerably different (16.1 and 38.3%) of our study (5.6%). In addition to the quantitative aspects, regardless of the extraction method applied it must take into account quality extract, in other words, the bio-activity of these active compounds. In (Küpeli *et al*., 2006) extraction time's was no reported.

In our study, by (ULTRA TURRAX, IKA^R WERKE) extraction method, we have accelerated the process of extracting and minimized the time of contact with the extract solvent, while preserving the bio-activity of its constituents. Similarly, cold this temperature, extraction flow along the exhaustion of the solvent at reduced pressure, allows obtaining the maximum of compounds and preventing their denaturing, or likely change due to the temperatures used in other methods of extraction.

But, without, forgetting the difference between our solvent for extractions and those of (Küpeli *et al*., 2006).

Concerning the level of phenolic compounds as in methanol extract as in acetone extract (46.72±4.69 and 55.80±2.75 mg PE/g DW) was superior to those measured in leaves by Meot-Duros *et al*., (2008) (16.44±0.30 mg GA/g DW).

Because of the multiple ways in which an antioxidant can protect biological molecules against oxidative damage, we measured different reactions to assess antioxidant activity, so as to determine the true antioxidant potential of *Eryngium maritimum* roots (Aruoma, 2003).

The antioxidant activity of each extract studied by us has not been reported before. Literature survey indicated antioxidant activity for *Eryngium maritimum* leaves using the (ABTS and TAC) assay (Meot-Duros *et al*., 2008).

Our study demonstrated the higher activity of butanol extract. The strong antioxidant property of this extract is associated to their phenols including flavonoids. This family from polyphenols have long been considered beneficial for health originally their good effects were thought to be due to their "antioxidative" effect and also their radical scavenging ability (Zhang and Björn, 2009). The scavenging activity of flavonoids depends to a high degree on their structures and physicochemical properties (Al-Bagieh and Weinberg, 1988).

Moreover, the antioxidant efficiency of flavonoids is directly related to their degree of hydroxylation is decreased in the presence of a sugar moiety. Flavonoids are effective scavengers hydroxyl and peroxyl radicals and of the superoxide anion (Bors *et al*., 1990; Chen *et al*., 1990).

Results of antibacterial activity of *Eryngium maritimum* leaves in (Meot-Duros *et al*., 2008) presented a strongest activity against two of the three *Pseudomonas* species tested (*Pseudomonas aeruginosa* and *Pseudomonas fluorescens*). Indeed, its 2 fractions had low MIC: 1 and 2 µg/mL polar and apolar fractions, respectively. Also, Apolar fraction inhibited *Staphylococcus aureus* and *bacillus cereus*. Conversely, *Listeria monocytogenes* and *Escherichia coli* were the most resistant bacteria to this 2 fraction.

In our study, results of antibacterial activity showed that *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus* and *bacillus cereus* were inhibited by all the extract. Thus, only methanol and butanol extract was active against *Pseudomonas aeruginosa*.

The antibacterial activity of plant extracts can be attributed not only to a single bioactive principle but also in concert action with other compounds (Sunayana *et al*., 2003). A number of phytochemicals have been studied for their antimicrobial activity and found potentially useful against infectious diseases. The chemical structure of the antimicrobial agents found in higher plants belong to most commonly encountered classes of higher plants secondary metabolites such as flavonoids (Watchter *et al*., 1999) and phenolic acids (Fernandez *et al*., 1996).

The antifungal activity of *Eryngium maritimum* roots studied by us has not been reported before.

CONCLUSION

The results of the present study suggest that *Eryngium maritimum* roots contain a considerable amount of phenolic compounds and had a significant antioxidant, antibacterial and antifungal activity. More detailed studies on chemical composition of this plant, as well as other *in vivo* assays are essential to characterize them as biological antifungal drug, antibiotic and antioxidant which are beyond the scope of this study.

REFERENCES

Al-Bagieh, N.H. and E.D. Weinberg, 1988. Benzylisothiocyanate: A possible agent for controlling dental caries. Microbios. Lett., 39: 143-151.

Andrew, C., 2001. Encyclopedia of Medicinal Plants. 2nd Edn., Dorling Kindersiey Ltd., London.

Antal, D.S., 2010. Medicinal plants with antioxidant properties from Banat Region (Romania): A rich pool for the discovery of multi-target phytochemicals active in free-radical related disorders. Analele Universităţii din Oradea-Fascicula Biologie, 17(1): 14-22.

Aruoma, O.I., 2003. Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant food. Mutat. Res., 523(524): 9-20.

Baytop, T., 1999. Therapy with Medicinal Plants in Turkey-Past and Present. 2nd Edn., Nobel Tip Basimevi, Istanbul, Turkey.

Bekkara, A.F., 1999. Study of Chemical Signals involved in the symbiosis of Vicia jaba and *Rhizobium leguminosarum*. Ph.D. Thesis, Es-Science, Biologie Végétale, Faculté des Sciences, Univ. ABB., Tlemcen, Algérie.

Berghe, V.A. and A.J. Vlietinck, 1991. Screening methods for Antibacterial and Antiviral Agents from Higher Plants. In: Mbosso, E.J.T., S. Ngouelaa, J.C.A. Nguediac, V.P. Bengc, M. Rohmer and E. Tsamo, (Eds.,) 2010. *In vitro* Antimicrobial Activity of Extracts and Compounds of Some Selected Medicinal Plants from Cameroon. J. Ethnopharmacology, 128: 476-481.

Bhengraj, A.R., S.A. Dar, G.P. Talwar and A. Mittal, 2008. Potential of a novel polyherbal formulation BASANT for prevention of Chlamydia trachomatis infection. Int. J. Antimicrob Agents, 32: 84-88.

Bors, W., W. Heller, C. Michel and M. Saran, 1990. Flavonoids as antioxidants: Determination of radical-scavenging efficiencies. Methods Enzymol, 186: 343-355.

Brand-Williams, W., H.E. Cuvelier and C. Berset, 1995. Use of a free radical method to evaluate antioxidant activity. Food Sci. Technol., 82: 25-30.

Cesquini, M., M.A. Torsoni, G.R. Stoppa and S.H. Ogo, 2003. t-BuOH-induced oxidative damage in sickle red blood cells and the role of flavonoids. Biomed. Pharmacother, 57: 124-129.

Chen, Y.T., R.L. Zheng, Z.J. Jia and Y. Ju, 1990. Flavonoids as superoxide scavengers and antioxidants. Free Radic. Bio. Med., 9: 19-21.

Cragg, G.M., D.J. Newman and K.M. Snader, 1997. Natural products in drug discovery and development. J. Nat. Prod., 60: 52-60.

Diaz, M.N., B. Frei and J.F. Keaney, 1997. Antioxidants and atherosclerotic heart disease. N. Engl. J. Med., 337: 408-416.

Djeridane, A., M. Yousfi, B. Nadjemi, D. Boutassouna, P. Stocker and N. Vidal, 2006. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chem., 97: 654-660.

Erdelmeier, C.A.J. and O. Sticher, 1986. Acyclohexenone and a cyclohexadienone glycoside from *Eryngium campestre*. Phytochemistry, 25: 741-743.

Fernandez, M.A., M.D. Garcia and M.T. Saenz, 1996. Antibacterial activity of the phenolic acid fractions of *Scrophularia fruescens* and *Scrophularia sambucifolia*. J. Ethnopharmacol., 53: 11-14.

Halliwell, B., 2000. The antioxidant paradox. Lancet, 355: 1179-1180.

Heinecke, J.W., 2003. Oxidative stress: New approaches to diagnosis and prognosis in atherosclerosis. Am. J. Cardiol, 91:12A-16A.

Ksouri, R., H. Falleh, W. Megdiche, N. Trabelsi, B. Mhamdi, K. Chaib, A. Bakrouf, C. Magné and C. Abdelly, 2009. Antioxidant and antimicrobial activities of the edible medicinal halophyte *Tamarix gallica* L. and related polyphenolic constituents. Food Chem. Toxicol., 47: 2083-2091.

Küpeli, E., M. Kartal, S. Aslan and E. Yesilada, 2006. Comparative evaluation of anti-inflammatory and antinociceptiactivity of Turkish *Eryngium* species. J. Ethnopharmacol., 107: 32-37.

Maxwell, S.R.J., 1995. Prospects for the use of antioxidant therapies. Drugs, 49: 345-361.

Meot-Duros, L., G. Le Floc and C. Magné, 2008. Radical scavenging, antioxidant and antimicrobial activities of halophytic species. J. Ethnopharmacol., 116(2): 258-262.

Murray, P.R., E.J. Baron, M.A. Pfaller, F.C. Tenover and R.H. Yolke, 1995. Manual of Clinical Microbiology. 7th Edn., ASM, Washington, DC., pp: 1773.

NCCLS., 1997. In Performance Standards for Antimicrobial Disk Susceptibility Test. Sixth Approved Standard, M2-A6, Wayne, PA.

Newman, D.J., G.M. Cragg and K.M. Snader, 2003. Natural Products as sources of new drugs over the period 1981-2002. J. Nat. Prod., 66: 10221037.

Oyaizu, M., 1986. Studies on products of the browing reaction prepared from glucose amine. Jpn. J. Nutr., 44: 307-315.

Quezel, P. and S. Santa, 1963. Nouvelle Flore De l'Algérie et Des Régions Désertiques Méridionales. Tome 2, Edn., Du Centre National de la Recherche Scientifique, France.

Singleton, V.L. and J.A. Rossi, 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic., 16: 144-158.

Smid, E.J. and L.G.M. Gorris, 1999. Natural Antimicrobials for Food Preservation. In: Shaur Rahman, M., (Ed.), Handbook of Food Preservation, Marcel Dekker, New York, pp: 285-308.

Suhaj, M., 2006. Spice antioxidants isolation and their antiradical activity. J. Food Compos. Anal., 19: 531-537.

Sunayana, V., A. Vadivukkarasi, T. Rajendran, X. Francis and E. Natarajan, 2003. Antibacterial potential of *Plectranthus amboinicus* (Lour) Spreng. A study *in vitro*. J. Swamy Botanic. Club, 20: 55-58.

Tereschuck, M.L., M.V.Q. Riera, G.R. Castro and L.R. Abdala, 1997. Antimicrobial activity of flavonoid from leaves of *Tagetes minuta*. J. Ethnopharmacol., 56: 227-232.

Urquiaga, I. and F. Leighton, 2000. Plant polyphenol antioxidants and oxidative stress. Biol. Res., 33(2): 55-64.

Vuuren, S.F.V. and D. Naidoo, 2010. An antimicrobial investigation of plants used traditionally in southern Africa to treat sexually transmitted infections. J. Ethnopharmacol., 130: 552-558.

Watchter, G., A. Hoffmann, T. Furbacher, M.E. Blake and B.N. Timmerman, 1999. Antibacterial and antifungal flavanones from *Eysenhardtia texana*. Phytochem., 52: 1469-1471.

Zhang, W.J. and L.O. Björn, 2009. The effect of ultraviolet radiation on the accumulation of medicinal compounds in plants. Fitoterapia, 80: 207-218.