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HPLC-DAD profiling and antioxidant activity of the *n*-butanol extract from aerial parts of Algerian *Crithmum maritimum* L.

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Abstract: *In this study, phenolic compounds from Crithmum maritimum L. n-butanol soluble fraction were quantified and identified spectrophotometrically and by using HPLC-DAD technics. They equally investigated for their antioxidant potential utilizing six in vitro assays: DPPH$^{\bullet}$, ABTS$^{\bullet+}$, $O_2^{\bullet-}$, Bleaching of β-carotene in linoleic acid, CUPRAC and Ferric reducing power. High amounts of total phenolics and flavonoids were recorded: 161.57± 0.479 µg GA_{eq}.mg^{-1} and 31.56± 0.291 µg Q_{eq}.mg^{-1} respectively. Nine compounds among them hydroxicinnamic acid and hydroxybenzoic acid derivatives, coumarins and flavonoids were identified. Chlorogenic acid known for their various pharmacological properties was detected as major compound of the extract. Rutin, vanillin, trans-2-hydroxycinnamic acid, ellagic acid, ferrulic acid, 6,7 dihydroxy coumarin, methyl 1,4 benzoquinone and trans-cinnamic acid were also detected. The extract was found to exhibit strong antioxidant capacities in all systems. Based on these results, it is right to conclude the n-butanol extract is promising source of natural antioxidants.*

Key words: *Crithmum maritimum* L.; HPLC-DAD; phenolics; flavonoids; antioxidant activity**.**

Introduction

Living organism produces free radicals spontaneously and in an excessive way, which conduct to the oxidative stress that would cause numerous pathological damages like cancer, neurodegenerative, cardiovascular and autoimmune diseases [1-4]. Natural antioxidants are group of substances able to enhance the organism's defense system. Vitamins, terpenes, alkaloids and phenolics isolated from medicinal plants were largely investigated to find natural antioxidants [5]. Phenolics are one of the most groups of phytochemicals studied in recent times due to their high and various biological activities. Phenolic acids, flavonoids, coumarins, stilbens, lignans, tanins and more of other phenolic compounds have been found to exhibit properties as anti-hypertensive, anti-inflammatory, antiproliferative, anticarcinogenic, antimicrobial and antioxidants [6- 8].

Crithmum maritimum L. belongs to the family Apiaceae, largely used as food ingredient and in traditional medicine in many countries since old times [9]. It mainly known as sea fennel, crest marine, marine fennel, rock samphire and sampier, a halophile aromatic plant grows wild on maritime rocks and occasionally on sands [10]. It is mostly distributed from Mediterranean and Black sea to the European

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Atlantic coast [9, 11]. In Algeria, the genus *Crithmum* is represented by only the species *C.maritimum* L. [12]. Researches on this species demonstrated the presence of essential oils [11], phenolic compounds [13-16], Vitamins and minerals [17, 18], proteins and amino acids [19] and fatty acids [20].

The soluble phenolic constituents of the *n*-butanol fraction from *C. maritimum* L. have not been studied previously. The present research aimed to identify and quantified phenolic components present in the *n*-butanol extract from aerial parts of *C.maritimum* L. in full flowering stage using Folin Ciocalteu, aluminum chloride and HPLC-DAD analysis and to determine their antioxidant potential by different methods: $DPPH^{\bullet}$, $ABTS^{\bullet+}$ and $O_2^{\bullet-}$ scavenging, lipid peroxidation, ferric reducing power and CUPRAC.

Material and methods

Plant material

Aerial parts of marine fennel (*Crithmum maritimum* L.) were collected in August 2016 at full flowering stage, in the region of Jijel North eastern of Algeria (36°47′16.33″ N Lat., 5°38′53.45″ E Long. and 3 m altitude). A voucher specimen (ZA / 144) was deposited at the laboratory of Biomolecules and Plant Breeding, Life Science and Nature Department, University of Larbi Ben Mhidi Oum El Bouaghi, Algeria.

Preparation of the extract

The Powdered aerial parts of *C. maritimum* L. (50 g) were macerated with 80 % ethanol at room temperature for three times every 24 h and filtered to obtain hydro alcoholic extract, which was concentrated under reduced pressure (40°) to remove ethanol, and then successively extracted with increasing polarity solvents; *n*-hexane, chloroform, ethyl acetate and *n*-butanol. Obtained extracts were stored at 4°C until analysis.

Phytochemical analysis

Total phenolics and flavonoids Quantification

The colorimetric method using Folin Ciocalteu as reagent was followed to determine total soluble phenolics amount in the *n*-butanol extract from *C. maritimum* L. aerial parts [21]. The reaction mixture was constituted with 500 µL of extract at 250 µg/mL, 2500 µL of F.C reagent (1/10 in water) followed by 2000 µL of (20 g/l) sodium carbonate. After ninety minutes, the absorbance was measured at 760 nm. The concentration of phenolics was found using the equation of linear regression obtained from the standard calibration curve:

$Y= 0.017x + 0.080$ Gallic acid (µg), $R^2 = 0.996$.

Flavonoid content of the extract was performed according to the method of aluminum trichloride showed by Miliauskas et al [22]**.** One milliliter of extract at 250µg/ml dissolved in methanol was added to one milliliter of 2% methanol solution of $AlCl_3$. The absorbance was read at 430 nm after incubation of ten minutes at ambient temperature. The concentration of flavonoids was calculated using the equation of linear regression obtained from quercetin curve:

$Y= 0.042x + 0.037$ quercetin (µg), $R^2 = 0.998$.

HPLC-DAD screening of phenolics

Analysis of butanol extract and 27 standard phenolics were carried out using a Shimadzu reverse phase high performance liquid chromatography (Shimadzu Cooperation, Japan) system that consist of a Shimadzu model LC-20AT solvent delivery unit and a Shimadzu model SPD-M20A diode array detection system and were controlled by LC-solution software (CBM-20A System Controller Shimadzu). The column temperature was set at 35 °C. The chromatographic separation was performed on a Inertsil ODS-3 (4µm, 4.0 mm x 150 mm) column and Inertsil ODS-3 guard column, mobile phases were aqueous acetic acid 0.1% (A) and methanol (B). Gradient elution from 2% to 100% was performed as previously showed by Barros et al. [23] and Tel-Çayan et al. [24]. Stock solution of sample was prepared in methanol at 8 $mg.mL^{-1}$ and filtered with an Agilent 0.45 µm filter. The injected volume was 20 µL. Detection was carried out diode array detector (DAD) using 254 nm wavelength. The detected phenolics were characterized by comparison of their retention times and results were expressed as micrograms per gram of dry weight.

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Determination of antioxidant activity

All tests were performed in 96-wells microplates using a (Perkin Elmer, Enspire) microplate reader**.** The results were established as 50% inhibition concentration ($IC_{50,}$ µg/mL) that means sample concentration providing 50 % activity (DPPH, ABTS, $O_2^{\bullet-}$ and β-Carotene-linoleic acid) and $A_{0.5}$ which corresponding the concentration indicating 0.500 absorbance (CUPRAC and Reducing power).

DPPH Free Radical Scavenging assay

The effect of the *n*-butanol extract and standard antioxidants (BHA and BHT) on 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was estimated according to the method of Blois [25]. Sample (40 µl; 6.25–200 $\mu g.ml^{-1}$) was added to 160 µl of DPPH methanol solution (10^{-4} M) in triplicate. The mixture was incubated for 30 min at room temperature, and then the absorbance was measured against a blank (methanol) at 517 nm. The percentage of DPPH scavenging effect was calculated using the equation:

$\%I= [(A_1- A_2)/A_1] \times 100$

Where; A_1 and A_2 are respectively absorbance of negative control and sample.

ABTS Cation Radical Scavenging Assay

Butanol extract was tested against ABTS cation radical using the method of Re et al. [26]. Briefly, 7 mM ABTS solution in water was prepared, and then 2.45 mM potassium persulfate was added to generate ABTS cation radical, stored in the obscurity at room temperature for 12h. The absorbance of this solution was adjusted with H_2O to 0.700 ± 0.020 at 734 nm. In every well of microplate, 160 µL of this solution was added to 40 µL of sample and standards in triplicate. Methanol was used as negative control while BHT and BHA were used as standard antioxidants. The absorbance was read at 734 nm after 10 min of incubation at room temperature and inhibition percentage was then calculated according to the DPPH formula.

β-Carotene-linoleic acid assay

The method of **Marco** [27] was used to evaluate the β-carotene-linoleic acid bleaching activity of our extract. Briefly, 0.5 mg of β-carotene was dissolved in 1 mL of chloroform, mixed with 25 µL of linoleic acid and emulsified by 200 µL of Tween 40. After evaporation of chloroform under vacuum at 40°C, the residue was recuperated with 100 mL of distilled water saturated with oxygen. Absorbance was adjusted to (0.800 - 0.900) at 470 nm with oxygenated water. To 160 µl of this solution, 40 µL of sample at concentrations (6.25 – 200) µg/mL and standards (BHA and BHT) was added. After incubation of two hours at 50 °C, absorbance was measured at 470 nm and the bleaching ratio of β-carotene was determined as follows:

$R = (ln\ a_1/a_2)/t$

Where; a_1 and a_2 were respectively absorbance at start of reaction (t_0) and after 120 min (t_{120}). Whereas, antioxidant activity (A %) was expressed as a percentage and calculated by the equation as bellow:

$A\% = [(R_{control} – R_{sample})/ R_{control}] * 100$

Superoxide anion radical scavenging assay

$O_2^{\bullet-}$ scavenging activity was measured by the alkaline DMSO method which monitored by Elizabeth and Rao [28] with some modification. This method is based on reducing Nitroblue tetrazolium (yellow color) into formazan (blue color) under generated alkaline DMSO. 130 µl of alkaline DMSO (prepared with dissolving 20 mg of NaOH in 100 mL of DMSO to generate $O_2^{\bullet-}$) was mixed with 40 µL of extract at different concentrations (6.25-200) µg/mL, and then add 30 µL of NBT (1 mg/mL in distilled water). α-tocopherol was used as standard and methanol was used as negative control. Absorbance was read at 560 nm after 10 min of incubation at room temperature, and percentage of $O_2^{\bullet-}$ inhibition was measured as shown with DPPH equation.

Reducing power assay

The reductive abilities of the extract were assessed using Fe^{3+} to Fe^{2+} reductive capacity as described by Oyaizu [29]. 10 µL of sample (1.562-50) µg/mL was mixed with 40 µL of phosphate buffer (pH 6.6) and 50 µL of potassium ferricyanide (1%) (1 g of $K_3Fe(CN)_6$ in 100 mL H_2O), and then the mixture was incubated at 50°C for 20 min. 50 µL of trichloroacetic acid (10%), 40 µl H_2O and 10 µl ferricchloride

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$FeCl_3$ (0.1%) were added to the mixture. Absorbance was measured at 700 against a blank (methanol). Results were given as absorbance and compared with those of BHT and BHA used as positive controls.

Cupric reducing capacity (CUPRAC)

The cupric reducing antioxidant capacity was determined using the method of Apak et al. [30] modified by Öztürk et al. [31] A mixture constituted for 60 µL ammonium acetate buffer (1M, pH 7.0), 50 µL of 7.5 mM neocuproin and 50 µL 10 mM (Cu Cl_2, $2H_2O$) was prepared and then 40 µL of sample solution at concentrations (6.25-200) µg/mL was added to the initial mixture. After one hour of incubation at room temperature, the absorbance at 450 nm was read against a blank reagent. Standard antioxidants used in this test were BHT and BHA. Results were given as absorbance and $A_{0.5}$ (µg/mL) values corresponding to the concentration indicating 0.50 absorbance intensity were assessed.

Statistical Analysis

All data and results were performed in triplicate and expressed as mean ± standard deviation (S.D).

Results

Total phenolics and flavonoids contents

According to the results shown in table (1), the amounts of total phenolics and flavonoids of *n*-butanol extract of *C.maritimum* L. were found to be important values with 161.57± 0.479 micrograms of gallic acid equivalent per mg of extract (µg $GA_{eq}.mg^{-1}Ext$) and 31.56± 0.291 micrograms of quercetin equivalent per mg of extract (µg $Q_{eq}.mg^{-1}Ext$). The extraction yield was determined to be 0.96 % in dry weight of plant.

Table 1. Determination of extraction yield, total phenolics and flavonoids contents in the *n*-butanol extract of *C. maritimum* L.

	Extraction yield (%)	Total phenolics (µg $GA_{eq}.mg^{-1}Ext$) [a]	Total flavonoids (µg $Q_{eq}.mg^{-1}Ext$) [b]
n-butanol extract	0.96	161.57± 0.479	31.56± 0.291

Results are expressed as means ± standard deviation of three measurements.

(a) Microgram of Gallic Acid Equivalent per milligram of extract.

(b) Microgram of Quercetin Equivalent per milligram of extract.

Identification and quantification of compounds by HPLC-DAD

Nine compounds including phenolic acids, flavonoids and coumarins were identified and quantified as figured in table (2). The most abundant compound detected in the *n*-butanol extract of *C.maritimum L. was* Chlorogenic acid (120.69 µg/g), followed by Rutin (30.44 µg/g), vanillin (6.04 µg/g), *trans*-2-hydroxycinnamic acid (5.76 µg/g), ellagic acid (3.01µg/g) and ferrulic acid (2.58 µg/g). Moreover, some compounds like 6,7 dihydroxycoumarin, Methyl 1,4 benzoquinone and *trans*-cinnamic acid were also noted in traces.

Table **2.** HPLC-DAD analysis of the *n*-butanol extract of *C.maritimum* L.

N°	Compounds	Retention time (min)	composition (µg/g)
1	Methyl 1,4 benzoquinone	09.65	0.68
2	6,7 dihydroxy coumarin	11.62	0.23
3	Vanillin	14.89	6.04
4	Chlorogenic acid	17.05	120.69
5	Ferrulic acid	19.76	2.58
6	*trans*-2-hydroxycinnamic acid	21.98	5.76
7	Rutin	22.01	30.44
8	Ellagic acid	22.54	3.01
9	*trans*-cinnamic acid	24.52	0.01

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Antioxidant activity

The results for antioxidant potential with six methods based on several mechanisms of action were represented in figure 1, as inhibition percentage as a function of concentration. The table 3, summarizes values of IC_{50}, $A_{0.5}$ and percentage of inhibition at minimum concentration. Trough all graphs, the activity was increasing dose-dependent way at concentrations from 6.25 to 200 µg/ml. At minimum concentration, the *n*-butanol extract of *C.maritimum* L. show an interesting antioxidant activity compared with those of standard antioxidants in all tests. In DPPH$^{\bullet}$, ABTS$^{\bullet+}$, $O_2^{\bullet-}$ and β-carotene tests; the extract gave values of IC_{50} proximate to those given by the standards. On the other hand, in CUPRAC and Ferric reducing power tests; values of $A_{0.5}$ were more important than had BHT.

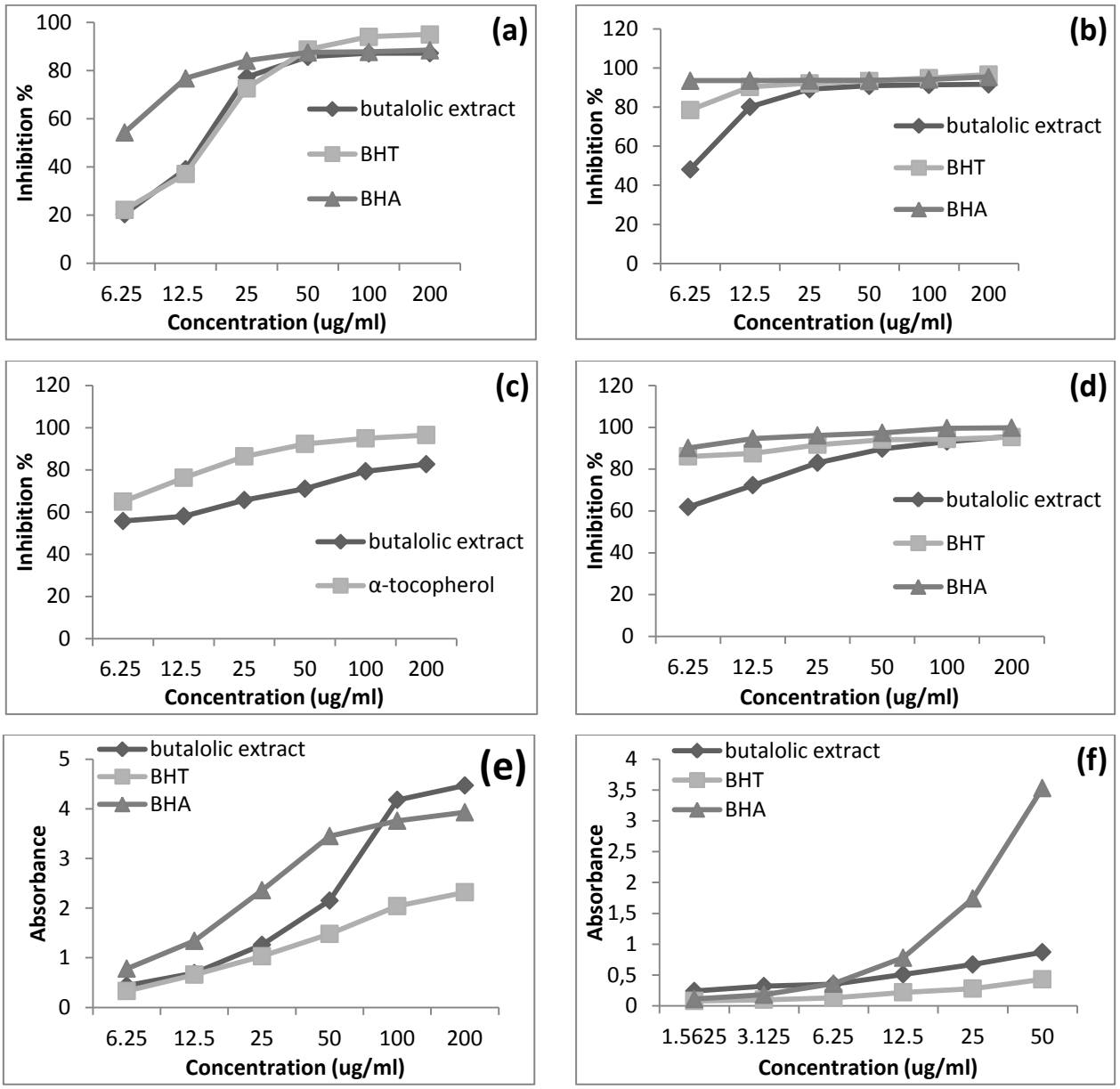

Fig. 1. Antioxidant activity; DPPH$^{\bullet}$ scavenging **(a)**, ABTS$^{\bullet+}$ scavenging **(b)**, $O_2^{\bullet-}$ scavenging **(c)**, Bleaching of β-carotene in linoleic acid **(d)**, CUPRAC **(e)** and Ferric reducing power **(f)** of *C.maritimum* L. butanolic extract.

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Assay		butanol extract	BHA	BHT	α-Tocopherol
DPPH$^{\bullet}$	I(%) at 6.25µg/mL	20.42±0.54	54.33±1.59	22.21±1.30	nt
	IC_{50} µg/mL	16.07±0.39	5.73±0.41	12.99±0.41	nt
$ABTS^{\bullet+}$	I(%) at 6.25µg/mL	48.18±1.06	93.52±0.09	78.55±3.43	nt
	IC_{50} µg/mL	6.94±0.40	1.81±0.10	1.29±0.30	nt
β-carotene-linoleic acid	I(%) at 6.25µg/mL	61.85±2.82	90.11±0.68	86.09±1.04	nt
	IC_{50} µg/mL	1.87±0.03	0.90±0.02	1.05±0.01	nt
$O_2^{\bullet-}$	I(%) at 6.25µg/mL	55.72±3.04	nt	nt	64.92±1.01
	IC_{50} µg/mL	3.46±0.28	nt	nt	3.19±0.40
CUPRAC	Abs at 6.25µg/mL	0.44±0.00	0.78±0.01	0.33±0.04	nt
	$A_{0.50}$ µg/mL	8.13±0.39	3.64±0.19	9.62±0.87	nt
Ferric reducing power	Abs at 6.25µg/mL	0.35±0.03	0.36±0.04	0.13±0.02	nt
	$A_{0.50}$ µg/mL	11.94±0.47	8.41±0.67	>50	34.93±2.38

Values expressed are means ± S.D. of three parallel measurements, nt: not tested., BHA: Butylated-hydroxyl anisole, BHT: Butylated-hydroxyl toluene

Discussion

Phenolics are qualitatively and quantitatively one of the broadest groups of secondary metabolites described in literature, they are ubiquitous in green plants and are highly diversified [32]. The Apiaceae family is well known by its richness in phenolic compounds [33]. Aerial parts from *C.maritimum* L. were good extracted by polar solvent *n*-butanol and gave good amounts of total phenolics and flavonoids. The quantification of these compounds was well documented in previous studies about polar fractions (methanol and ethanol extracts) of the plant from aerial parts, separated organs, the same part of plant in different growing stages and the plant from different localities [13-16].

A diversified mixture of phenolic components was identified in the *n*-butanol extract from hydroxicinnamic acids, hydroxybenzoic acids and coumarins to flavonoids. It has been noted that chlorogenic acid was the main component of *n*-butanol extract, which were in accordance with Nabet et al. [14] who found a concentration of 6366 mg/Kg dw in the hydro-methanolic extract from aerial parts of the Algerian variety (Bejaia). In a study carried out by Generalic Mekinic et al [15] on separated organs from Croatian variety during flowering stage, concentration of chlorogenic acid in flowers, stems and leaves was estimated at 7.7 ± 1.3, 0.7 ± 0.0 and 8.1 ± 0.1 mg/g of extract respectively. More recently, Generalic Mekinic et al [16] reported an amount of 5646.5 ± 110.2 µg/g of dry plant for chlorogenic acid in the ethanol extract from aerial parts at full flowering stage. The presence of chlorogenic acid in relatively high amounts in the plant kingdom as well as in species belonging to Apiaceae family was largely described in literature [15, 33, 34,]**.** There are many reports on biological activities of phenolic acids, coumarins and flavonoids, chlorogenic acid and derivatives have multiple properties including anti-inflammatory [06], antispasmodic [35], inhibition of the HIV-1 integrase [36], antibacterial and antioxidant [07]. In the same context, therapeutic potencies of flavonoids and coumarins as antioxidant, cardiovascular disease

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protection, vascular fragility, antinflammatory, neurodegenerative diseases prevention and anti-platelet aggregation were widely published [8].

The antioxidant activity was assessed using scavenging properties, chain breaking, reducing potential and redox assays. The *n*-butanol extract of *C.maritimum* L. was found to exhibit a strong antioxidant potential in all assays. Otherwise, moderate activity was reported by authors about methanol and hydromethanol extracts [13, 14, 16, 37, 38]. The antioxidant effect of the extract might be attributed to the high amount of phenolics especially chlorogenic acid as predominant active compound and also rutin, vanillin, *trans*-2-hydroxycinnamic acid, ellagic acid and ferrulic acid.

Conclusion

The *n*-butanol extract from aerial parts of *C. maritimum* L. with high amounts of soluble phenolics predominantly with chlorogenic acid as hydroxycinnamic acid derivative and other compounds with variable concentrations including rutin, vanillin, *trans*-2-hydroxycinnamic acid, ellagic acid and ferrulic acid was found to have good antioxidant activity obviously trough scavenging several radicals, inhibiting of lipid peroxidation and reducing abilities. Also, this effect may be due to the synergistic action of present compounds. Our findings were very encouraging and motivating to pursue supplementary investigations in order to isolate and purify individual components that are conducting this act and further more to study *in vivo* biological activities.

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References

[1] Aruoma, O. I., Free radicals, oxidative stress, and antioxidants in human health and disease, *J. Am. Oil Chem.* Soc. **1998**, *75*, 199–212.

[2] Montaigner, L.; Olivier, R.; Pasquier, C., Oxidative stress in Cancer. AIDS and neurodegenerative deseases, Eds., Marcel Dekker, New York, 157, **1998**.

[3] Riboli, E.; Norat, T., Epidemiologic evidence of the protective effect of fruit and vegetables on cancer risk, *Am. J. Clin. Nutr.* **2003**, *78*, 559–569.

[4] Letelier, M.E.; Molina-Berríos, A.; Cortés-Troncoso, J.; Jara-Sandoval, J.; Holst, M.; Palma, K.; Montoya, M.; González-Lira, V., DPPH and oxygen free radicals as pro-oxidant of biomolecules, *Toxicol. Vitro.* **2008**, *22*, 279–286.

[5] Li, A. N.; Li, S.; Li, H. B.; Xu, D. P.; Xu, X. R.; Chen, F., Total phenolic contents and antioxidant capacities of 51 edible and wild flowers, *J. Funct. Foods*, **2014**, *6*, 319–330.

[6] Dos Santos, M.; Almeida, MC.; Lopes, N. P.; De Souza, G. E. P., Evaluation of the Anti inflammatory, Analgesic and Antipyretic Activities of the Natural Polyphenol Chlorogenic Acid, *Biol. Pharm. Bull.* **2006**, *29*, 2236-2240.

[7] Cheng, J.; Dai, F.; Zhou, B.; Yang, L.; Liu, Z., Antioxidant activity of hydroxycinnamic acid derivatives in human low density lipoprotein: mechanism and structure-activity relationship, *Food Chem.* **2007**, *104*, 132–139.

[8] Raffa, D.; Maggio, B.; Raimondi, M. V.; Plescia, F.; Daidone, G., Recent discoveries of anticancer flavonoids, *Eur. J. Med. Chem.* **2017**, *142*, 213-228.

[9] Coiffard, L., Aperçu historique sur l'utilisation médicinale et alimentaire du crithme maritime, *Revue d'histoire Pharmaceutique*, **1991**, *38*, 313-317.

[10] Renna, M.; Gonnella, M., The use of the sea fennel as a new spice-colorant in culinary preparations, *Int. J. Gastron. Food Sci.* **2012**, *1*, 111-115.

[11] Males, Z.; Blazevic, N.; Plazibat, M., Variations in the yield and composition of the essential oil of *Crithmum maritimum* L., *Acta. Pharm.* **2001**, *51***,** 81–84.

[12] Quezel P. ; Santa, S., Nouvelle flore de l'Algérie et des régions désertiques méridionales, Eds. C.N.R.S, Paris, **1963**.

[13] Meot-Duros, L.; Le Floch, G.; Magne, C., Radical scavenging, antioxidant and antimicrobial activities of halophytic species, *J. Ethnopharmacol*. **2008**, *116*, 258–262.

Corresponding author: saber.boutellaa@gmail.com

DOI: 10.2478/asn-2019-0002

[14] Nabet, N.; Boudries, H.; Chougui, N.; Loupassaki, S.; Souagui, S.; Burló, F.; Hernández, F.; Carbonell-Barrachina, A. A.; Madani K.; Larbat, R., Biological activities and secondary compound composition from *Crithmum maritimum* aerial parts, *Int. J. Food Prop*. **2017**, *20*, 1843-1855.
[15] Generalic Mekinic, I.; Blazevic, I.; Mudnic, I.; Burcul, F.; Grga, M.; Skroza, D.; Jercic, I.; Ljubenkov, I.; Boban, M.; Miloš, M.; Katalinic, V., Sea fennel (*Crithmum maritimum* L.): phytochemical profile, antioxidative, cholinesterase inhibitory and vasodilatory activity, J. *Food Sci. Technol*. **2016**, *53*, 3104-3112.
[16] Generalić Mekinić, I.; Šimat, V.; Ljubenkov, I.; Burčul, F.; Grga, M.; Mihajlovski, M.; Lončar, R.; Katalinić, V.; Skroza, D., Influence of the vegetation period on sea fennel, *Crithmum maritimum* L. (Apiaceae), phenolic composition, antioxidant and anticholinesterase activities, *Ind. Crop. Prod*. **2018**, *124*, 947-953.
[17] Franke, W., Vitamin C in sea fennel (*Crithmum maritimum*), an edible wild plant, *Econ. Bot.* **1982**, *36*, 163-165.
[18] Coiffard L.; De Roeck-Holtzhauer, Y., *Crithmum maritimum* L. (*Apiaceae*): Hydromineral composition and phytochemical screening, *Ann. Pharm. Fr.* **1994**, *52*, 153–159.
[19] Coiffard L.; De Roeck-Holtzhauer, Y., Geographical and seasonal variations in protein content and free aminoacids of *Crithmum maritimum* L. (*Apiaceae*), *Acta. Bot. Gallica*, **1995**, *142***,** 405-414.
[20] Guil-Guerrero, J. L.; Rodriguez-Garcia, I., Lipids classes, fatty acids and carotenes of the leaves of six edible wild plants, *Eur. Food Res. Technol.* **1999**, *209*, 313-316.
[21] Singleton V. L.; Rossi, J.A.J., Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, *Amer. J. Enol. Viticult*. **1965**, *16*,144-158.
[22] Miliauskas, G.; Venskutonis P.R.; Beek, T.A., Screening of radical scavenging activity of some medicinal and aromatic plant extracts, *Food Chem*. **2004**, *85*, 231-237.
[23] Barros, L.; Duenas, M.; Ferreira, I.C.F.R.; Baptista P.; Santos-Buelga, C., Phenolic acids determination by HPLC–DAD–ESI/MS in sixteen different Portuguese wild mushrooms species, *Food Chem. Toxicol*. **2009**, *47*, 1076-1079.
[24] Tel-Çayan, G.; Öztürk, M.; Duru, M. E.; Rehman, M. U.; Adhikari, A.; Türkoglu A.; Choudhary, M. I., Phytochemical investigation, antioxidant and anticholinesterase activities of *Ganoderma adspersum*, *Ind. Crop. Prod*. **2015**, *76*, 749-754.
[25] Blois, M. S., Antioxidant determinations by the use of a stable free radical, *Nature*, **1958**, *181*, 1199-1200.
[26] Re, R.; Pellegrini, A.; Proteggente, A.; Pannala, M.; Yang M.; Rice-Evans, C., Antioxidant activity aplying an improved ABTS radical cation decolorization assay, *Free Radic. Biol. Med.* **1999**, *26*, 1231-1237.
[27] Marco, G.J., A rapid method for evaluation of antioxidants, *J. Am. Oil Chem. Soc.* **1968**, *45*,594-598.
[28] Elizabeth K.; Rao, M.N.A.,Oxygen radical scavenging activity of *curcumin*, *Int. J. Pharm.* **1990**, *58*, 237-240.
[29] Oyaizu, M., Studies on products of browning reactions: antioxidative activities of browning reaction prepared from glucosamine, *Jap. J. Nutr.* **1986**, *44*, 307–315.
[30] Apak, R.; Güçlü, K.; Özyürek M.; Çelik, S. E., Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity) assay, *Microchim. Acta*. **2008**, *160*, 413–419.
[31] Öztürk, M.; Kolak, U.; Topçu, G.; Öksüz S.; Choudhary M.I., Antioxidant and anticholinesterase active constituents from *Micromeria cilicica* by radical-scavenging activity-guided fractionation, *Food Chem.* **2011**, *126*, 31–38.
[32] Havsteen, H.B., The biochemistry and medical significance of the flavonoids, *Pharmacol. Ther.* **2002**, *96*, 67-202.
[33] Pandey, M. M.; Vijayakumar, M.; Rastogi S.; Rawat, A.K.S., Phenolic content and antioxidant properties of selected Indian spies of Apiaceae, *J. Herbs Spices Med. Plants*, **2012**, *18*, 246-256.
[34] Upadhyay R.; Mohan Rao, L., An outlook on chlorogenic acidsoccurrence, chemistry, technology, and biological activities, *Crit. Rev. Food Sci. Nutr.* **2013**, *53*, 968–984.
[35] Farah, A.; De Paulis, T.; Trugo L. C.; Martin, P. R., Effect of roasting on the formation of chlorogenic acid lactones in coffee, *J. Agric. Food Chem.* **2005**, *53*, 1505-1513.
[36] Robinson, W. E.; Reinecke, M. G.; Abdel-Malek, S.; Jia Q.; Chow, S. A., Inhibitors of HIV-1 replication that inhibit HPV integrase, *Proc. Natl. Acad. Sci. USA*. **1996**, *93*, 6326-6331.

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[37] Houta, O.; Akrout, A.; Neffati M.; Amri, H., Phenolic contents, antioxidant and antimicrobial potentials of *Crithmum maritimum* cultivated in Tunisia arid zones, *J.B.A.P.N*. **2011**, *1*, 138-143.
[38] Jallali, I.; Zaouali, Y.; Missaoui, I.; Smeoui, A.; Abdelly C.; Ksouri, R., Variability of antioxidant and antibacterial effects of essential oils and acetonic extracts of two edible halophytes: *Crithmum maritimum* L. and *Inula crithmoïdes* L., *Food Chem.* **2014**, *145*, 1031- 1038.

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