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Lipid Composition and Antioxidant Activities of *Daucus maritimus* Seeds

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Abstract: *Daucus maritimus* seeds were extracted successively with hexane, dichloromethane, ethyl acetate and ethanol in a soxhlet apparatus to provide four oily extracts named, respectively: HE, DM, EA and ET. The seed oils were analyzed for fatty acids, sterols, some physicochemical characteristics and antioxidant activities. The results of the analyses revealed that all oils contain an appreciable amount of unsaturated fatty acids (76.12-90.28%). The oleic acid was the dominating fatty acid in HE (62.07%), DM (73.16%) and EA (80.48%) oils whereas the petroselinic acid dominates in the ET oil (77.31%). The dominant saturated acid was palmitic acid. The oils were also found to contain high levels of β-sitosterol (up to 67.90% of total sterols) and stigmasterol (up to 38.84% of total sterols). The antioxidant potential of all extracts was evaluated by radical scavenging activity using 2,2-diphenyl-1-picryl hydrazyl (DPPH) assay and β-carotene bleaching method.

Key words: *Daucus maritimus*, seed oils, fatty acids, sterols, antioxidant activity

INTRODUCTION

As apart of our investigation into some medicinal plants known in Tunisia (Bouaziz *et al*., 2002; Ben Salah *et al*., 2002; Koubaa and Damak, 2003; Louati *et al*., 2003; Fakhfakh and Damak, 2007) and in order to continue present research on potential biological natural compounds, we report below the study of oil composition and antioxidant activity of *Daucus maritimus*.

Oil yielding crop plants are very important for economic growth of agricultural sector. The oilseeds containing unusual fatty acids are very important for industry; they can be used in pharmaceuticals, cosmetics, detergents, soaps, textiles, surfactants (Hosamani and Sattigeri, 2000). On the other hand, no oil from a single source has been found to be suitable for all purposes because oils from different sources generally differ in their composition. This requires the search for new sources of novel oils. Several plants are now growing, not only for food and fodder, but also for a striking range of products with an industrial application.

The genus *Daucus*, consisting of 14 species, belongs to the Umbelliferae family (Apiaceae). *Daucus maritimus* (Lam.) Batt. is a subspecies of *Daucus carota*. It is a biennial plant, with fleshy leaves. It is widespread in the Mediterranean region (L'abbé, 1983; Tutin *et al*., 1981). No report indicates its uses in traditional medicine, but relatively few phytochemical analyses have been published by Baranska *et al*. (2005) and Miladi *et al*. (2005). Furthermore, insufficient information is available regarding to the seeds content in this species.

The seeds were undertaken and extracted in a soxhlet apparatus successively with solvents of different polarities (hexane, dichloromethane, ethyl acetate and ethanol) to provide four oily extracts: HE, DM, EA and ET.

The aim of the present investigation is to determine the composition of *Daucus maritimus* seed oils and to evaluate their antioxidant activities by 2,2-diphenyl-1-picryl hydrazyl and β-carotene bleaching methods.

MATERIALS AND METHODS

Plant material: *Daucus maritimus* (Lam.) Batt. was collected in June 2005 at Sfax, Tunisia. It was identified by Pr. M. Chaieb (Département de Biologie, Faculté des Sciences, Université de Sfax, Tunisie). Voucher specimens (N° LCSN109) were deposited at the Laboratoire de Chimie des Substances Naturelles, Faculté des Sciences, Université de Sfax, Tunisie.

Extraction and physicochemical properties: Seed powder of *Daucus maritimus* (Lam.) Batt. (300 g) was extracted in a Soxhlet apparatus with hexane, dichloromethane, ethyl acetate and ethanol to give successively four oily extracts: HE, DM, EA and ET.

Some physicochemical indices of the four oils were determined. The following were evaluated according to the methods listed in the ISO (International Organization for Standardization) and in IOC (International Oil Council) : acid value (ISO 660, 1996); saponification value (ISO 3657, 1988); Iodine value (ISO 3961, 1996); unsaponifiable value (ISO 3596, 1996); peroxide value (IOC 3960, 1998).

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Fatty acid analysis: The fatty acids methyl esters were prepared by adding 10 mL of sodium methoxide solution (0.25 M) to 1 g of oil. The mixtures were refluxed for 15 min, followed by adding 15 mL of acid methanolic solution (1M) and refluxed again for 10 min according to the procedure reported by ISO 5509. The solutions were cooled by adding 20 mL of water and the fatty acids methyl esters were extracted three times with 20 mL of hexane and concentrated under reduced pressure. Then, the fatty acid composition of the four oils was determined on a Hewlett-Packard GC/MS (HP6890-HP5973 MSD-Agilent Technology, Wiling, USA) equipped with a HP-5 column (30 m× 0.25 mm i.d.) under the following operating conditions: oven temperature: 150°C for 4 min, rising to 290°C at 3°C min^{-1} and held for 10 min; injector temperature: 250°C; scanning scope: 50-600 amu; ionization voltage: 70 eV; ionization electric current: 30 μA.

Sterol composition: Unsaponifiable matter of *Daucus maritimus* seed (DMS) oils was extracted and determined in oils samples according to IOC (COI/T.20/Doc.No. 10, 2001). One gram of each oil was refluxed with 10 mL of 10% ethanolic potassium hydroxide for 1 h. The reaction mixture was diluted with 10 mL of distilled water and the unsaponifiable matter was extracted three times with 20 mL portion of ether. The ether extracts were combined and washed three times with distilled water until they became alkali-free. The ether extract was dried over anhydrous sodium sulphate and evaporated. The sterol fractions obtained by TLC separation from the unsaponifiables of the oils were derivitised with silanizing mixture (pyridine-hexamethyl disilazane-trimethyl chlorosilane 9:3:1, v/v/v) in the ratio of 50 μL for every mg of sterols.

Further determination of the sterols was carried out on a Hewlett-Packard GC/MS (HP6890-HP5973 MSD-Agilent Technology, Wiling, USA) equipped with a HP-5 column (30 m × 0.25 mm i.d.) under the following operating conditions: oven temperature: 150°C for 1 min, rising to 260°C at 10°C min^{-1} and held for 10 min, then to 270°C at 2°C min^{-1} and held for 10 min, then to 300°C at 5°C min^{-1}; injector temperature: 280°C; carrier gas: helium (1 mL min^{-1}); scanning scope: 50-600 amu; ionization voltage: 70 eV; ionization electric current: 30 μA.

Scavenging activity on DPPH radical: The antioxidant activity of the DMS extracts was assessed by the scavenging effect on DPPH radical (2,2-diphenyl-1-picrylhydrazyl) (Brand-Williams *et al*., 1995; Chen *et al*., 1999; Naik *et al*., 2003). Briefly, 1.5 mL of DPPH solution (10-5 M, in 95% Ethanol) was incubated with 1.5 mL of DMS extract at varying concentrations (0.1-5 mg). The reaction mixture was shaken well and incubated in the dark for 30 min at room temperature. The absorbance of each sample was measured at the spectrophotometer (Bibby Anadéo) at 517 nm against a blank of EtOH.

The radical scavenging activity (RSA) was measured as a decrease in the absorbance of DPPH and was calculated using the following equation:

$$\text{Scavenging effect (\%)} = [1 - \frac{A_{Sample(517nm)}}{A_{Control(517nm)}}] \times 100$$

The extract concentration providing 50% inhibition (EC_{50}%) was calculated from the graph of Scavenging effect percentage against the extract concentration. BHT was used as a reference compound. Tests were carried out in triplicate.

β-carotene bleaching test: The oxidative losses of β-carotene/linoleic acid emulsion were used to assess the anti-oxidation ability of the DMS extracts (Chevolleau *et al*., 1992; Moure *et al*., 2000). Ten milligram of β-carotene (type 1 synthetic, Sigma, St. Louis, MO, USA) was dissolved in 10 mL of $CHCl_3$ and 0.2 mL of the solution was placed into a boiling flask containing 20 mg linoleic acid and 200 mg Tween-40 (Sigma). After removal of $CHCl_3$, 50 mL of oxygenated distilled water was added to the flask and vigorously shaken to form an emulsion. An absorbance at 470 nm was immediately recorded after adding 2 mL of the sample to the emulsion, which was regarded as t = 0 min. The round-bottomed flasks were stoppered and placed in an incubator at 50°C. The absorbance at 470 nm was determined every 15 min for 120 min. A second emulsion, consisting of 20 mg of linoleic acid and 200 mg of Tween 40 and 50 mL of oxygenated water, was also prepared and served as blank to zero the spectrophotometer. The antioxidant activity coefficient (AAC) was calculated according to the following equation:

$$AAC = [(A_{A(120)} - A_{C(120)}) / (A_{C(0)} - A_{C(120)})] \times 1000$$

where, $A_{A(120)}$ is the absorbance of the antioxidant after 120 min, $A_{C(120)}$ is the absorbance of the control after 120 min and $A_{C(0)}$ is the absorbance of the control at 0 min. The AAC was calculated from the graph of absorbance against time. BHT was used as a reference compound. Tests were carried out in triplicate.

RESULTS AND DISCUSSION

Physicochemical properties: Table 1 presents the yields and some physico-chemical characteristics of DMS oils.

Table 1: Physicochemical properties of *Daucus maritimus* seed (DMS) oils

Extracts	HE	DM	EA	ET
Yield (%)	5.4±0.3	5.3±0.2	2.1±0.3	2.0±0.3
Acid value (mg KOH g^{-1})	13.5±0.5	10.3±0.5	23.1±0.5	17.9±0.5
Iodine value	250.8±0.5	233.4±0.6	271.9±0.6	289.9±0.3
Peroxide value (meq kg^{-1})	261.2±1.7	51.2±1.7	93.75±1.7	13.7±1.7
Saponification value (mg KOH g^{-1})	117.2±0.8	153.1±0.8	129.6±0.8	109.4±0.8
Unsaponifiable matter (%)	44.3±0.3	38.3±0.7	23.6±0.5	36.7±0.7

Values are Means±SD of three determination

Table 2: Fatty Acid Compositions of *Daucus maritimus* oils

Fatty acid	HE (%)	DM (%)	EA (%)	ET (%)
Myristic (C14)	0.42	0.24	-	-
Palmitic (C16)	10.24	8.58	8.48	13.26
Linoleic (C18:2)	13.22	11.22	9.82	9.43
Petroselinic (C18:1)	-	-	-	77.31
Oleic (C18:1)	62.07	73.16	80.48	-
Stearic (C18)	2.23	1.93	1.22	-
Cis-oleïc (C18:1)	0.83	2.74	-	-
Arachidic (C20)	0.55	0.37	-	-
Behenic (C22)	0.44	0.44	-	-
Lignoceric (C24)	0.69	0.25	-	-
Cerotic (C26)	0.80	-	-	-
Montanic (C28)	2.38	-	-	-
Melissic (C30)	3.32	-	-	-
Saturated fatty acids	21.07	11.57	9.70	13.26
Unsaturated fatty acids	76.12	87.12	90.28	86.73

-: Not detected

All oils show high saponification values suggesting their use in production of liquid soap and shampoos. The high levels of acid value indicate that these oils could only be recommended for industrial use. The four oils samples have high iodine value, thus reflecting a high degree of unsaturation and placing them in the drying group oils similar to Linseed and Conophor oils (Akpuaka and Nwankwor, 2000). These iodine values were confirmed by the fatty acid composition of the oils presented in Table 2.

Fatty acids: Fatty acid composition of the oils and their percentages are presented in order of their elution on the column in Table 2. All samples were characterized by high amounts of unsaturated fatty acids (76.12-90.28%). Oleic acid was the main unsaturated fatty acids in HE (62.07%), DM (73.16%) and EA (80.48%) oils whereas petroselinic acid prevails in ET oil (77.31%). The major saturated fatty acid in these oils was palmitic acid (8.48-13.26%).

The presence of a very high level of unusual petroselinic acid in ET oil is of interest as this compound could be used as a plastic lubricant, in the manufacture of nylons and for cosmetics. Moreover, petroselinic acid is used in treatment of inflammations.

The results obtained in this study showed that the fixed oils from DMS are rich in polyunsaturated fatty acids which play an important role in industry. Therefore, it would be interesting to know more about the possible pharmacological effects of this specie.

Sterol composition: In almost all plant fats and oils, sterols are the most quantitatively important unsaponifiable components. Cholesterol was detected in DM, EA and ET oils in a small amount (0.33-1.28%). We have observed the presence of campesterol in the DM, EA and ET oils. The main components for all oils were β-sitosterol (54.91-67.90%) and stigmasterol (26.43-38.84%). Two unknown peaks were detected, one eluted after cholesterol in HE oil (3.26%) and the other one eluted after Δ^7-sitosterol in DM oil (0.82%). Further work is in progress to identify these peaks (Table 3).

Scavenging activity on DPPH radical: The RSA of the DMS extracts was assessed using an ethanolic solution of the stable free radical, DPPH. A freshly prepared DPPH solution exhibited a deep purple color with a maximum absorption at 517 nm. This purple color disappears when an antioxidant is present in the medium. Thus, antioxidant molecules can quench DPPH free radicals and convert them into a colorless product, resulting in a decrease in absorbance at 517 nm. The RSA values of hexane, dichloromethane, ethyl acetate and ethanol extracts of DMS are presented in Fig. 1. The results are expressed as the ratio percentage of sample absorbance decrease and the absorbance of DPPH solution in the absence of an extract at 517 nm. RSA% was proportional to the concentration of the extract. EC_{50} values (concentration

Table 3: Relative retention times and mass spectra of the sterols in DMS oily extracts

		Sterol yields (%)				
Sterols	RR_t	HE	DM	EA	ET	MS-data of sterol-TMS ether derivative
Cholesterol	0.79	-	1.28	0.46	0.33	M^+ 458, 368, 353, 329, 281, 255, 247, 159, 129, 73.
Unknown	0.83	3.26	-	-	-	M^+ 458, 368, 353, 329, 281, 267, 253, 247, 143, 129, 73.
Campesterol	0.89	-	3.00	2.14	1.57	M^+ 472, 457, 382, 367, 343, 281, 261, 255, 129, 73.
Stigmasterol	0.93	26.43	30.19	34.84	38.84	M^+ 484, 469, 394, 379, 355, 255, 213, 159, 129, 83.
β-sitosterol	1.00	67.90	55.96	54.91	54.92	M^+ 486, 471, 396, 381, 357, 275, 255, 213, 129, 73.
Sitostanol	1.01	-	1.49	1.26	1.03	M^+ 488, 473, 431, 398, 383, 306, 305, 281, 215, 75.
Δ^5-avenasterol	1.02	-	1.05	-	-	M^+ 484, 394, 386, 379, 371, 355, 298, 281, 257, 255, 253, 129, 73, 55.
Δ^7-sitosterol	1.07	2.41	6.21	6.38	4.00	M^+ 486, 471, 429, 398, 381, 345, 303, 281, 255, 229, 213, 207, 75.
Unknown	1.09	-	0.82	-	-	M^+ 469, 386, 371, 343, 296, 281, 253, 213, 207, 75.

-: Not detected. RR_t : Relative Retention time to β-sitosterol (26.80 min) using a HP-5 30 m column

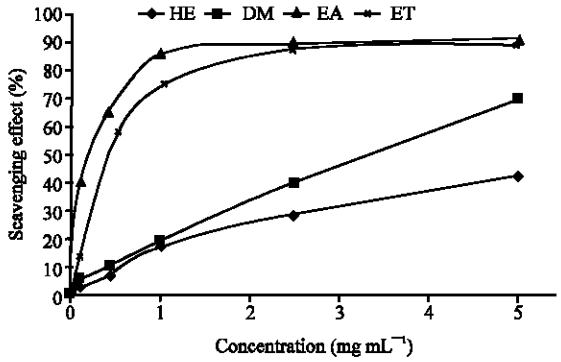

Fig. 1: Scavenging activity (%) on DPPH radicals of DMS oils

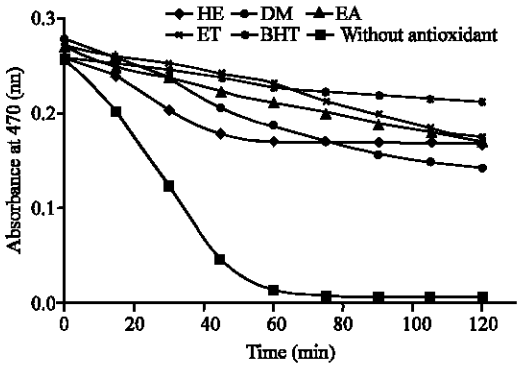

Fig. 2: Change of absorbance at 470 nm with time for DMS oils (2 mg mL) in β-carotene/linoleic acid emulsion

Table 4: Comparison of antioxidant properties of DMS oily extracts and BHT

Oily extracts	EC_{50} (μg mL^{-1})	AAC
HE	-	648
DM	3333	545
EA	194	656
ET	472	667
BHT	69	822

of sample required to scavenge 50% of free radicals) of DMS extracts and BHT are indicated in Table 4. The EA extract has the greater scavenging effect followed by ET extract. From the analyses of Fig. 1, we can conclude that the scavenging effects of seeds extract increase as the concentration increases.

Antioxidant assay using the β-carotene bleaching method: The antioxidant activities of the DMS extracts were evaluated by the β-carotene bleaching method, in which the oxidation of linoleic acid takes place. Linoleic acid hydroperoxides attack the β-carotene molecule and as a result, it undergoes a rapid decolorization. The corresponding decrease in absorbance can be monitored spectrophotometrically. The presence of antioxidant extracts can hinder the extent of β-carotene bleaching by acting on the linoleate-free radical and other free radicals formed in the system (IENICA, 2000). Accordingly, the absorbance decreased rapidly in samples without antioxidant whereas, in the presence of an antioxidant, they retained their color and thus their absorbance, for a longer time. The absorbance of the emulsion decreased with time (Fig. 2). DMS extracts, BHT showed a variant anti-oxidation activity. The decreasing rate of absorbance for an emulsion sample with the added of hexane and ethyl acetate extracts was significantly lower than the samples with the addition of other extracts. The AAC are summarized in Table 4. The order of AAC at 2 mg mL^{-1} among the four extracts of DMS was as follows: ET extract > EA extract > HE extract > DM extract.

CONCLUSION

This study shows that *Daucus maritimus* seeds provide different oils containing a large amount of unsaponifiable matter. The physico-chemical characteristics and fatty acid composition of these oils suggest that they have some industrial potential. Furthermore, the ethanol extract could be used as alternative resource of petroselinic acid.

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