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PHYTOCHEMICALS, ANTIOXIDANT AND α-AMYLASE INHIBITORY ACTIVITIES OF *SMYRNIUM OLUSATRUM* L. LEAF, FLOWER AND FRUIT

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Abstract

Alexanders (Smyrnium olusatrum) has long been regarded as a food and medicinal plant. The essential oil composition of S. olusatrum leaf, flower and fruit was characterized by high proportion of furanosesquiterpenes (51.66-69.35%). Furthermore, quantitative differences among plant parts were observed for the majority of oil components. Among all organs significant variability in fatty acids composition were also observed. Main constituents were found to be polyunsaturated fatty acids (37.74-49.36%).
The total phenolic, flavonoid and carotenoid contents varied significantly between plant parts. Flower extract exhibited the highest contents of total phenolic (48.97 mg GAE/g) and flavonoid (52.63 mg RE/g). The β-carotene and lycopene contents were in the range of 4.55-26.14 mg/100g, and 8.00-49.45/100g, respectively.
Methanolic extracts and essential oils of different organs were found to possess antioxidant activities, as determined by scavenging effect, chelating activity and β-carotene-linoleic acid model system. Extracts and essential oils showed a strong inhibitory activity against α-amylase. However, the level of biological activity varied according to extracts and organs.

Keywords: *Antioxidant activity, Type 2 diabetes, carotenoid, furanosesquiterpenes, polyunsaturated fatty acids, Polyphenols, Smyrnium olusatrum.*

INTRODUCTION

The genus *Smyrnium* L. (Umbelliferae) consists of seven species widely distributed throughout the world (Mölleken et al., 1998; Mungan et al., 2011). The majority of *Smyrnium* species are known for their diuretic, depurative, aperients, carminative, restorative and stomachic properties (Mölleken et al., 1998; Khanahmadi et al., 2010). Young sprouts, plant stems and roots are consumed as vegetables, which impart a pleasant flavour similar to celery (Mölleken et al., 1998).

In Tunisia, the genus includes two species (*Smyrnium olusatrum* L. and *Smyrnium perfoliatum* (L.) Mill. ssp. *Rotundifolium* (D.C.) P.F.) which grow wild in different bioclimatic zones (Pottier-Alapetite, 1981).

Smyrnium olusatrum L., commonly known as Alexanders, is a stout, glabrous, celery-scented, biennial plant. Leaves are 2-3-pinnate, dark green and glossy. Flowers are yellow, shortly pedicellate and grouped in numerous umbels. Fruit is a schizocarp comprising two single-seeded mericarps, broadly ovoid and laterally compressed.

In traditional medicine, *Smyrnium olusatrum leaves are used as* antiscorbutic, fruits as stomachic and antiasthmatic, and roots are known for their aromatic, appetite stimulant, diuretic and laxative properties (Mölleken et al., 1998; Papaioannou et al., 2010).

Previous phytochemical studies on *Smyrnium olusatrum* root, stem, leaf and fruit have revealed the presence of a wide array of components such as sesquiterpene lactones, furanosesquiterpenes and phenolic acids (Mölleken et al., 1998; El-Gamal, 2001). However, there is no information regarding carotenoids, fatty acids and antioxidant activity of essential oils and extracts of different plant parts.

Therefore, the aim of this study was to determine the essential oil and fatty acids compositions, phenolic and carotenoid contents of *Smyrnium olusatrum* leaf, flower and fruit, and examine for the first time the antioxidant and the α-amylase inhibition activities of the

essential oils and methanol extracts of these plant organs.

MATERIALS AND METHODS

Plant material

Smyrnium olusatrum aerial parts were harvested from a random sample of 25-30 plants growing wild in Korbous Jebel Mountain (Cap Bon region of Tunisia, latitude 36° 47′ 18″ N; longitude 10° 35′ 14″ E, altitude 400m, rainfall 550mm/year). Leaves, flowers and immature fruits were air-dried at room temperature for two weeks.

Essential oil extraction and GC-FID and GC-MS analyses

Three lots of 100 g of each organ type (leaves, flowers and fruits), finely ground in a mortar grinder mill, were separately hydrodistilled for 3 h using a Clevenger-type apparatus. The obtained essential oils were dried using anhydrous sodium sulphate and then stored at 4°C until analyses. The essential oil composition was determined by GC-FID and GC-MS analyses following the methods of Messaoud and Boussaid (2011). Essential oil components were identified by comparison of their retention indices determined with reference to a homologous series of C_9-C_{24} n-alkanes and with those of authentic standards. Identification was confirmed by comparison of their mass spectra with those recorded in NIST08 and W8N08 libraries. Component relative percentages were obtained directly from GC-FID peak areas without correction factors.

Lipid extraction, Fatty acid methyl esters (FAMEs) preparation and GC-FID analyses

Triplicate sub-samples of 10 g of each ground organ were separately extracted using the continuous Soxhlet extraction technique with petroleum ether for 3 h. Extracts were filtered and concentrated under reduced pressure and temperature.

FAMEs were prepared according to Lechevallier (1966). In a methylation tube, 0.2 ml of the concentrated extract of total lipid were saponified with 4 ml methanolic sodium hydroxide solution (0.5 M) for 15 min in a boiling water bath at 65 °C. As for transmethylation, the mixture was homogenized with 3 ml of BF_3 methanolic solution (14%), and the reaction was allowed to proceed for 5 min at 65 °C. Subsequently 10 ml of water were added to the mixture and FAMEs were extracted twice with 10 ml of petroleum ether.

FAMEs were analyzed by a gas chromatograph Agilent model 6980 series, equipped with HP-Innowax capillary column (30 m x 0.25 mm; 0.25 µm film thickness), FID (280 °C) and split/splitless injector (220 °C). Oven temperature was held at 150°C for 1 min, then heated to 200°C at a rate of 15°C/min, and from 200 to 250°C at 2°C/min, and held isothermally at 250°C for 10 min. Helium was the carrier gas at an initial flow rate of 1 ml/min. Split ratio was 20:1. The identification of FAMEs peaks was determined by a comparison of their relative retention times with those of FAME authentic standards. Quantification of fatty acid methyl esters, expressed as percentages, was obtained directly from the GC peak area integration.

Polyphenols extraction

The air-dried leaf, flower and fruit were finely ground separately in a mortar grinder mill. Triplicate sub-samples of 1g of each ground organ were extracted with 20 ml of pure methanol for 24 h in a water bath shaker maintained at room temperature. The extracts were filtered using a 0.45-µm Millipore filter and stored in a brown bottle at 4°C prior to further analysis.

Total phenolic and flavonoid contents

Total phenol contents were determined using the Folin-Ciocalteu method (Singleton and Rossi, 1965). Total phenolic contents of leaf, flower and fruit were expressed in terms of milligrams gallic acid equivalent per gram of dry weight (mg GAE/g DW). Analyses were carried out in triplicate.

Flavonoid contents of leaf, flower and fruit were estimated according to the aluminum chloride colorimetric method of Djeridane et al. (2006). Flavonoid contents were expressed as milligrams of rutin equivalent per gram of dry weight (mg RE/g DW).

Carotenoid contents

A fine dried sample (150 mg) of each organ type (leaves, flowers and fruits) was vigorously shaken with 10 ml of acetone-hexane mixture (4:6) for 1 min and then filtered through Whatman filter paper. The absorbance of the obtained filtrate was measured at 453, 505, 645 and 663nm. β-carotene and lycopene contents were calculated according to the following equations: β-carotene (mg/100ml) = 0.216 x

A663 - 1.22 x A645 - 0.304xA505 + 0.452 xA453; lycopene (mg/100ml) = -0.0458 x A663 + 0.204 x A645 - 0.304 x A505 + 0.452 x A453, and further expressed in mg/100 g of dry weight (Nagata and Yamashita, 1992).

Free radical-scavenging activity

The DPPH radical scavenging activity was measured from the bleaching of purple colored methanol solution of DPPH radical according to Brand-Williams et al. (1995). Briefly, 50 µl of each methanol extract or essential oil (at different concentrations in methanol) were mixed with 1.95 ml of 60 µM DPPH radical solution and allowed to react in the dark for 30 min. The absorbance was determined at 517 nm. Radical-scavenging activity was estimated as RSA% = $[(A_0 - A_1)/A_0]$ x100, where A_0 is the absorbance of the control reaction and A_1 is the absorbance of the test sample. The antiradical activity was presented by IC_{50} value, defined as the concentration of the antioxidant required to scavenge 50% of DPPH present in the test solution. Trolox was used as positive control.

Ferrous ion chelating activity

The ferrous ion chelating activity of different organ extracts and essential oils was measured according to Yan et al. (2006). 0.5 ml of different concentrations of extracts or essential oils in methanol were added to 0.5 ml of $FeSO_4$ solution (0.125 mM), and left for incubation at room temperature for 5 min. Then, the reaction was initiated by adding 0.5 ml of ferrozine (0.3125 mM). The mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was measured at 562 nm against a methanol blank. The ability to chelate ferrous ion was calculated using the following formula: Chelating effect (%) = [100 x (A_C - A_S/A_C)]; where A_C is the absorbance of the control and A_S represents the absorbance of the tested sample. Results were expressed as IC_{50} (efficient concentration corresponding to 50% ferrous iron chelating). EDTA was used as positive control.

Inhibition of β-Carotene bleaching

A solution of β-carotene was prepared by dissolving β-carotene (2 mg) in chloroform (20 ml). 4 ml of this solution was mixed with 40 mg linoleic acid and 400 mg Tween 40. After the chloroform was removed at 40 °C under vacuum, 100 ml of oxygenated ultra-pure water was added, and then the emulsion was vigorously shaken. Aliquots (1.5 ml) of this emulsion were transferred into different test tubes containing different concentrations of organ extracts or essential oils in methanol (0.1 ml). As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm and the test emulsion was incubated in a water bath at 50 °C for 120 min, when the absorbance was measured again. In the negative control, the tested sample was substituted with ultra-pure water. β-carotene bleaching inhibition was calculated using the following equation: Inhibition (%) = $[(A_t-C_t)/(C_0-C_t)]$ x100; where A_t and C_t are the absorbance values measured for the test sample and control, respectively, after incubation for 120 min, and C_0 is the absorbance values for the control measured at zero time during the incubation. The results are expressed as IC_{50} values, the concentration required to cause a 50% β-carotene bleaching inhibition. BHT was used as positive control.

α-amylase inhibition assay

Mathanolic extracts were concentrated to dryness. The resulted extract or essential oils were dissolved in DMSO [50% in sodium phosphate buffer (0.02 M, pH 6.9)] to give different concentrations.

α-amylase inhibition was tested by the agar disc diffusion method, according to Cha et al. (2009). Starch hydrolysis assay was performed on plates composed of 1% (w/v) starch dispersed in 1.5% agar. Sterile Whatman disc papers (6 mm) were individually placed on agar plates, and 10 µl of samples (porcine pancreatic α-amylase with or without extracts) were applied to the filter paper disc. After incubation at 37 °C for 72 h, starch plates were stained with iodine solution (5 mM I_2 in 3% KI) for 15 min at room temperature. Iodine was removed from the plates by washing with distilled water. Amylase activity was determined by measuring the zone diameter of hydrolysed areas around the wells. The percentage of inhibition of α-amylase was calculated using the following formula: Amylase inhibition (%) = 100 x (d0 - d1)/d0; where d0 is the diameter of the negative control, and d1 is the diameter of the tested sample.

Statistical analysis

For each organ type, three samples were analyzed and all assays were carried out in triplicate. Results were presented as means ± standard deviation (SD). For each measured data, quantitative differences between organs was assessed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test using the SAS v. 9.1.3 program. The level of statistical significance was set at $p \leq 0.05$.

RESULTS AND DISCUSSIONS

Essential oil composition

Essential oil compositions of *S. olusatrum* leaf, flower and fruit were summarized in Table 1. Twenty three components, representing 94.51%, 95.66% and 96.32% of the total essential oil, were identified in leaves, flowers and fruits, respectively.

Significant variations in the oil composition were observed between *S. olusatrum* plant parts. Furanosesquiterpenes (65.41%) and sesquiterpene hydrocarbons (19.65%) formed the predominant fractions of leaf essential oil. The main compound was curzerene (30.98%), followed by furanoeremophil-1-one (28.61%) and germacrene D (9.02%). Flower oil showed the highest percentage of myrcene (8.23%), curzerene (42.23%), furanodiene (6.81%), germacrone (12.78%), heneicosane (1.97%) and tricosane (3.74%), and was found to be rich in furanosesquiterpenes (51.66%), monoterpene hydrocarbons (21.43%), oxygenated sesquiterpene (12.78%) and sesquiterpene hydrocarbons (9.84%).

Table 1. Chemical composition (%) of essential oils of *Smyrnium olusatrum* plant parts

Compounds	**RI**	**Leaf**	**Flower**	**Fruit**
α-pinene	938	0.31 ± 0.02^{c}	1.41 ± 0.17^{b}	1.76 ± 0.12^{a}
β-Pinene	978	tr^{b}	tr^{b}	0.46 ± 0.2^{a}
Myrcene	991	1.02 ± 0.11^{c}	8.23 ± 0.73^{a}	2.51 ± 0.32^{b}
β-Phellandrene	1010	4.07 ± 0.28^{c}	6.08 ± 0.92^{b}	**9.89 ± 0.87^{a}**
Cis-β-Ocimene	1040	0.61 ± 0.10^{a}	tr^{b}	tr^{b}
Citronellyl acetate	1356	0.29 ± 0.06	tr	tr
β-Elemene	1387	1.55 ± 0.11	1.4 ± 0.22	1.65 ± 0.28
β-Caryophyllene	1418	2.05 ± 0.32^{a}	tr^{b}	tr^{b}
γ-Elemene	1429	0.48 ± 0.14^{a}	tr^{b}	0.42 ± 0.11^{a}
α-Humulene	1445	0.34 ± 0.09^{a}	tr^{b}	tr^{b}
Germacrene D	1474	**9.04 ± 0.74^{a}**	2.97 ± 0.13^{c}	3.78 ± 0.21^{b}
Curzerene	**1483**	**30.98 ± 1.58^{c}**	**42.23 ± 1.23^{a}**	**36.97 ± 1.75^{b}**
â-Bisabolene	1507	2.76 ± 0.22^{a}	1.89 ± 0.14^{b}	0.85 ± 0.11^{c}
δ-Cadinene	1516	0.38 ± 0.08^{a}	tr^{b}	tr^{b}
Germacrene B	1546	3.09 ± 0.21	3.58 ± 0.33	2.88 ± 0.17
Furanodiene	1678	5.49 ± 0.32^{b}	6.81 ± 0.27^{a}	5.62 ± 0.72^{b}
Germacrone	1682	2.84 ± 0.36^{b}	**12.78 ± 0.82^{a}**	2.76 ± 0.41^{b}
Furanoeremophil-1-one	1735	**28.61 ± 1.97^{a}**	2.62 ± 0.47^{b}	2.37 ± 0.29^{b}
Alexandrofuran	1739	tr^{b}	tr^{b}	0.57 ± 0.12^{a}
1-β-acetoxy-furanoeudesm-4 (15)-ene	1761	0.32 ± 0.11^{b}	tr^{b}	**23.71 ± 0.96^{a}**
1-β-acetoxyfuranoeudesm-3-ene	1763	tr	tr	0.11 ± 0.02
Heneicosane	2101	tr^{b}	1.97 ± 0.13^{a}	tr^{b}
Tricosane	2302	0.35 ± 0.14^{b}	3.74 ± 0.24^{a}	tr^{c}
Total identified (%)		94.51 ± 1.27	95.66 ± 1.84	96.32 ± 1.53
Monoterpene hydrocarbons (%)		6.36 ± 0.88^{c}	21.43 ± 1.85^{a}	14.62 ± 0.72^{b}
Oxygenated monoterpenes (%)		0.29 ± 0.06	tr	tr
Sesquiterpene hydrocarbons (%)		19.65 ± 0.93^{a}	9.84 ± 0.56^{b}	9.58 ± 0.62^{b}
Oxygenated sesquiterpenes (%)		2.84 ± 0.36^{b}	12.78 ± 0.82^{a}	2.76 ± 0.41^{b}
Furanosesquiterpenes (%)		65.41 ± 2.07^{b}	51.66 ± 1.88^{c}	69.35 ± 2.32^{a}

Values are given as mean ± SD; RI: retention indices relative to n-alkanes on a HP-5MS column;
tr: trace (< 0.1%).Values in each row followed by different letter are significantly different ($P < 0.05$).

A large proportion of the fruit essential oil was composed of furanosesquiterpenes (69.35%), monoterpene hydrocarbons (14.62%) and sesquiterpene hydrocarbons (9.58%). Curzerene

(36.97%), 1-β-acetoxy-furanoeudesm-4(15)-ene (23.71%) and β-phellandrene (9.89%) were detected to be the major components in the oil from fruits.

The variation of essential oil composition between *S. olusatrum* organs have been reported (Mölleken et al., 1998; Bertoli et al., 2004; Papaioannou et al., 2010). However, independently of the chemical polymorphism among organs, the essential oil composition of the species presented in our study differed from these earlier reports. This finding indicates that the essential oil composition of *S. olusatrum* is influenced by environmental and/or genetic factors.

Fatty acid composition

Thirteen fatty acid components, representing 91.13 to 92.16% of the total oil according to organ type, were identified (Table 2). All plant parts were found to be rich in palmitic acid (12.62-13.83), palmitoleic acid (3.87-12.19%), oleic acid (5.19-18.35%), linoleic acid (15.57-23.81%) and α-linolenic acid (22.17-26.22%). The high contents of unsaturated fatty acids, mainly linoleic and linolenic acids, in different organs could make *S. olusatrum* leaf, flower and fruit lipids an important source for a variety of healthy applications. Polyunsaturated are essential in the human diet since they cannot be synthesized by the body, and they are very important to human immumne system, to help regulate blood pressure and to alleviate cardiovascular, inflammatory, heart diseases, atherosclerosis, diabetes and other diseases (Simopoulos, 1991;Tapiero et al., 2002; Richard et al., 2009).

Significant quantitative variations in the fatty acid composition between *S. olusatrum* organs were disclosed (Table 2). Leaves contained the highest percentage of saturated fatty acids (36.12%) and the lowest amount of monounsaturated and polyunsaturated ones (17.61 and 37.74%, respectively). The highest contents of myristic acid (3.54%), palmitoleic acid (12.19%), stearic acid (7.84%), arachidic acid (2.58%), behenic acid (2.44%) and lignoceric acid (4.47%) were determined in leaves. Conversely, flowers and fruits were found to be significantly rich in oleic acid (15.81-18.35%), linoleic acid (23.14-23.81%) and α-linolenic acid (24.22-26.22%).

As far as our literature survey could as certain, there is only one report regarding the fatty acid composition of *S. olusatrum* leaf growing wild in Southern Italy (Conforti et al., 2011). This study reveals lower amounts of linoleic and linolenic acids than that observed in our work. The composition of fatty acids depends on genetic factors and geographical origin as well as on the used methodology for isolation and extraction (Primomo et al., 2002; Boschin et al., 2007).

Table 2. Fatty acid composition (%) of different *Smyrnium olusatrum* plant parts

Fatty acid	**Leaf**	**Flower**	**Fruit**
Myristic acid (C14:0)	3.54 ± 0.31^{a}	0.68 ± 0.15^{b}	0.56 ± 0.21^{b}
Palmitic acid (C16:0)	13.35 ± 0.98	13.83 ± 1.45	12.62 ± 1.29
Palmitoleic acid (C16:1)	12.19 ± 1.12^{a}	3.87 ± 0.22^{c}	7.12 ± 0.15^{b}
Stearic acid (C18:0)	7.84 ± 0.83^{a}	3.93 ± 0.25^{b}	3.52 ± 0.51^{b}
Oleic acid (C18:1)	5.19 ± 1.02^{c}	18.35 ± 0.93^{a}	15.81 ± 1.77^{b}
Linoleic acid (C18:2)	15.57 ± 1.53^{b}	23.81 ± 0.66^{a}	23.14 ± 1.03^{a}
α-Linolenic acid (C18:3)	22.17 ± 1.64^{b}	24.22 ± 1.35^{ab}	26.22 ± 1.88^{a}
Arachidic acid (C20:0)	2.58 ± 0.22^{a}	0.92 ± 0.15^{b}	0.68 ± 0.21^{b}
Eicosenoic acid (C20:1)	0.22 ± 0.11	0.15 ± 0.06	0.17 ± 0.04
Behenic acid (C22:0)	2.44 ± 0.34^{a}	0.82 ± 0.13^{b}	0.97 ± 0.19^{b}
Tricosanoic acid (C23:0)	0.14 ± 0.06	0.15 ± 0.03	0.17 ± 0.08
Lignoceric acid (C24:0)	4.47 ± 0.77^{a}	0.26 ± 0.10^{b}	0.28 ± 0.13^{b}
Cerotic acid (C26:0)	1.76 ± 0.23	1.18 ± 0.42	1.21 ± 0.36
Total identified (%)	91.46 ± 2.41	92.16 ± 2.17	91.13 ± 1.93
Saturated Fatty Acids	36.12 ± 1.82^{a}	21.77 ± 1.47^{b}	20.01 ± 1.71^{b}
Monounsaturated fatty acids	17.61 ± 1.61^{b}	22.37 ± 1.88^{a}	23.1 ± 1.01^{a}
Polyunsaturated fatty acids	37.74 ± 1.14^{b}	48.03 ± 1.79^{a}	49.36 ± 1.26^{a}

Values are given as mean ± SD. Values in each row followed by different letter are significantly different ($P < 0.05$).

Phenolic and carotenoid contents

There are considerable variations among phenolic and carotenoid contents for the three analyzed organs (Table 3). The total phenolic and flavonoid contents ranged from 31.48 to 48.97 mg GAE/g and 7.46 to 52.63 mg RE/g, respectively. Flowers contained the highest contents of total phenolic and flavonoids, followed by leaves and fruits.

A previous study on *S. olusatrum* leaf hydroalcoholic extract (Conforti et al., 2011) reported a different total phenolic content (70 mg chlorogenic acid equivalents per gram of extract) than that observed in our present study. However, there is no information regarding flowers and fruits.

To the best of our knowledge no data exist regarding the carotenoid contents in *Smyrnium olusatrum*. In our study, β-carotene and lycopene contents were determined by spectrophotometric methods. Significant differences in carotenoid concentration were also found among plant parts, and leaves exhibited the highest concentrations of β-carotene (26.14 mg/100 g DW) and lycopene (49.45 mg/100 g DW), followed by fruits and flowers (Table 3).

Phenolic and carotenoid compounds are secondary plant metabolites. The quantitative and qualitative variations of these phytochemical classes between plant parts have been largely determined. These natural products are marker of the nutritional quality of foods. Polyphenols and carotenoids are known for their antioxidant activity and possible beneficial roles in human health, protecting against oxidative damage to cells, stimulating immune function, reducing the risk of cancer and cardiovascular disease (Selappan et al., 2002; Barros et al., 2011).

Table 3. Total phenolic, flavonoid, ß-carotene and lycopene contents of *Smyrnium olusatrum* plant parts.

Organs	Total phenols	Flavonoid	ß-carotene	Lycopene
Leaf	33.22±1.19^{b}	22.36±1.13^{b}	26.14±1.92^{a}	49.45±2.03^{a}
Flower	48.97±1.92^{a}	52.63±1.09^{a}	4.55±0.68^{c}	8.00±0.97^{c}
Fruit	31.48±1.73^{b}	7.46±0.87^{c}	6.24±0.37^{b}	10.19±0.78^{b}

Values are given as mean ± SD. Total phenolic was expressed as mg gallic acid equivalent in 1 g of dry sample. Flavonoid was expressed as mg rutin equivalent in 1 g of dry sample. ß-carotene was expressed as mg in 100 g of dry sample. Lycopene was expressed as mg in 100 g of dry sample. Values in each column followed by different letter are significantly different ($P < 0.05$).

Antioxidant activity

The antioxidant activities of *S. olusatrum* extracts have never been measured. Therefore, three different in vitro assays were used for the evaluation of the antioxidant properties of methanolic extracts and essential oils of leaves, flowers and fruits. The results of scavenging activity on DPPH radicals, inhibition of β-carotene bleaching, and chelating ability are shown in Table 4. Methanolic extracts of different *S. olusatrum* parts gave statistically similar DPPH scavenging activity (IC_{50} = 0.126 mg/ml for leaf, IC_{50} = 0.092 mg/ml for flower, 0.138 mg/ml for fruit), while Trolox was a considerably more effective DPPH radical scavenger (IC_{50} = 0.076 mg/ml).

S. olusatrum flower extract exhibited the highest β-carotene bleaching inhibition (IC_{50}= 0.105 mg/ml) and chelating ability (IC_{50} = 2.84 mg/ml), than leaf and fruit methanolic extracts. However, all extracts exhibited low antioxidant activity when compared to that reported for standards BHT and EDTA (IC_{50} = 0.029 and IC_{50} = 0.019, respectively). The greatest antioxidant activity of flowers is in agreement with their higher phenolic and flavonoid contents in comparison to the other plant parts.

The essential oils were found to be less active than methanolic extracts. Although all oils showed no metal chelating activity, the free radical scavenging capacity, based on IC_{50} values, ranged from 40.68 mg/ml (flower) to 48.32 mg/ml (leaf). Essential oils of different plant parts (IC_{50} = 26.35 mg/ml for leaf, IC_{50} = 20.82 mg/ml for flower, and IC_{50} = 23.74 mg/ml for fruit) are also able to inhibit β-carotene bleaching. From the results, we can infer that the antioxidant effect was associated to furanosesquiterpenes which formed the predominant fractions of all essential oils.

Recent interest in these substances has been stimulated the potential health benefits arising from the antioxidant, antinociceptive, hypothermic, anti-inflammatory and antifungal activities of these furanosesquiterpenoids compounds (Amorim et al., 2009; Fraternale et al., 2011).

The antioxidant activity of essential oils cannot be attributed to the major compounds as minor compounds are likely to play a significant role in the activity, and synergistic effects being also reported (Kelen and Tepe, 2008). Phenols, but also many terpenes, notably monoterpenes, are known to exhibit antioxidant properties (Misharina et al., 2009). Accordingly, the highest antioxidant abilities of *S. olusatrum* flower essential oil were probably related to their richness in hydrocarbon monoterpenes and/or to the synergistic effect of more than one individual oil compound.

α-Amylase inhibition

Medicinal plants continue to play an important role in the treatment of diabetes. The inhibition of α-amylase activity, is considered to be an effective strategy for the control of diabetes by diminishing the absorption of glucose (Hara and Honda, 1990). α-amylase inhibitors have been one of the research hotspots as oral hypoglycemic agents for diabetic. As shown in Figure 1, degradation of starch by pancreatic α-amylase was inhibited by methanolic extracts and essential oils of all *S. olusatrum* organs (table 5). However, percentage inhibition varied according to type of extract (methanolic extract and essential oil) and plant parts. Flower methanolic extract (250µg/ml) displayed the highest inhibition (48.78%). The percentage inhibition of essential oil (2000µg/ml) varied between 13.41% (fruits) and 31.08% (flowers).

Table 4. Antioxidant activities of the essential oils and the methanolic extracts of *Smyrnium olusatrum* plant parts.

	DPPH (IC_{50}, mg/ml)	**â-carotene bleaching inhibition (IC_{50}, mg/ml)**	**Chelating ability (IC_{50}, mg/ml)**
Methanolic extract			
Leaf	0.126 ± 0.022^{c}	0.193 ± 0.009^{d}	4.36 ± 0.55^{a}
Flower	0.092 ± 0.012^{c}	0.105 ± 0.011^{e}	2.84 ± 0.37^{c}
Fruit	0.138 ± 0.016^{c}	0.291 ± 0.018^{c}	5.62 ± 0.21^{b}
Essential oil			
Leaf	48.32 ± 2.62^{a}	26.35 ± 2.41^{a}	-
Flower	40.68 ± 1.36^{b}	20.82 ± 1.75^{b}	-
Fruit	47.97 ± 2.44^{a}	23.74 ± 1.88^{ab}	-
Synthetic antioxidant			
Trolox	0.076 ±0.004^{d}		
BHT		0.029 ± 0.002^{f}	
EDTA			0.019 ±0.008^{d}

Values are given as mean ± SD. Values in each column followed by different letter are significantly different ($P < 0.05$).

Table 5.α-amylase inhibition essay of methanolic extract and essential oil of *Smyrnium olusatrum* organs

	Diameter of hydrolysed areas (mm)	
Organ	Methanolic extract (250µg/ml)	Essential oil (2000µg/ml)
Leaves	17.42 ± 0.83 (27.16% ± 1.22) *	16.43 ± 0.51 (31.38% ± 0.88)
Flowers	12.25 ± 0.75 (48.78% ± 1.97)	14.82 ± 0.46 (38.08% ± 1.02)
Fruits	18.10 ± 0.44 (24.32% ± 0.91)	20.71 ± 0.38 (13.41% ± 0.73)

Zone diameter of hydrolysed areas for the Control is 23.91±0.4 (using 1U/ml of α-amylase) and 16.24 ± 0.34 (using 0.75U/ml of α-amylase) *: Percentage of inhibition is in parenthesis

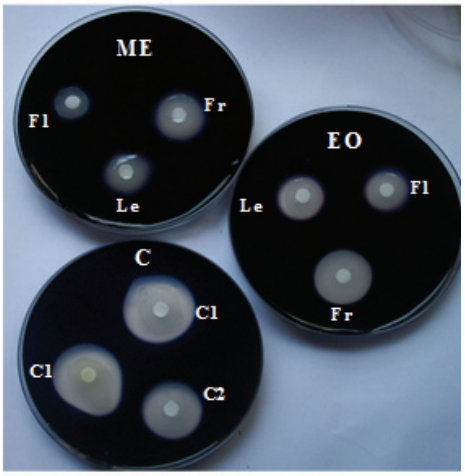

Figure 1. The inhibition of α-amylase by methanolic extracts and essential oils of *S. olusatrum* organs as detected by the agar diffusion method. C: negative control (C1:1U/ml; C2:0.75U/ml), ME: methanolic extract (Fl: flowers, Le: leaves, Fe: fruits), EO: essential oil (Fl: flowers, Le: leaves, Fe: fruits).

CONCLUSIONS

Natural products, especially those produced by medicinal plant species, are currently under special interest due to their safety, usefulness and accessibility. This study, on *Smyrnium olusatrum* plant parts, reports appreciable amounts of several interesting phytochemicals. According to the results of this study, the essential oil and the methanolic extracts of *S. olusatrum* may be suggested as a new potential source of bioactive compounds.

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