FACTA UNIVERSITATIS
Series: **Physics, Chemistry and Technology** Vol. 8, Nº 1, 2010, pp. 25 - 37
DOI: 10.2298/FUPCT1001025R

A NOTE ON THE VOLATILE SECONDARY METABOLITES OF *FOENICULUM VULGARE* MILL. (APIACEAE)[†]

UDC 577.13 : 635.75

Niko S. Radulović[*], Polina D. Blagojević

Department of Chemistry, Faculty of Science and Mathematics, University of Nis, Serbia

Abstract. *An analysis (GC and GC/MS) of Foeniculum vulgare Mill. (fennel) root and schizocarp essential oils and diethyl ether extracts enabled the identification of 89 different components, representing 98.2-100% of the total samples. One fourth of the identified compounds (24 in total) are reported for the first time as fennel volatiles. The most abundant classes of constituents of all four analyzed samples were the phenylpropanoids (69.5-85.5%) and monoterpenoids (11.7-26.9%). The dominant volatile metabolites of the schizocarps were fenchone (13.3-18.8%) and (E)-anethole (66.1-69.0%). Contrary to that, terpinolene (6.2-6.5%) and dillapiole (71.4-77.5%) were the major volatiles of fennel roots. The most striking differences between the chemical composition of the analyzed oils and their corresponding extracts were a rather surprising presence of a significant relative amount of apiole in the root oil (9.3%; this compound was detected only in trace amounts in the root extract) and the high percentage of 10-nonacosanone in the schizocarp extract (5.8%; this ketone was absent from the schizocarp oil). Possible explanations of the mentioned differences were put forward. The AMS (average mass scan of the total ion chromatogram) profiles/fingerprints of the volatile oils and extracts of F. vulgare roots and fruits are also given in the paper.*

Keywords: *Foeniculum vulgare Mill., Apiaceae, essential oil, diethyl ether extract, root, schizocarp*

1. INTRODUCTION

Foeniculum vulgare Mill. (fennel; *morač* in Serbian) is a well-known umbelliferous (Apiaceae) plant. It is native to southern Europe and the Mediterranean area. Fennel fruits (schizocarps) are rich in essential oil, with phenylpropanoid (*E*)-anethole being its major constituent. Besides this, monoterpenoid glycosides and phenolic compounds are also among the secondary metabolites of this plant species [1]. For centuries, fennel fruits have been used as a traditional herbal medicine in Europe and China and it has been

[†] Acknowledgment: This research was funded by the Ministry of Science and Technological Development of Serbia (Project 172061).
[*] Corresponding author. E-mail: nikoradulovic@yahoo.com.
Received December 6th 2010; revised January 13th 2011; accepted January 17th 2011.

known that their constituents are able to regulate menstruation, alleviate the symptoms of female climacteric syndrome, and increase libido [1, 2]. Investigations of *F. vulgare* extracts and essential oil, as well as of some compounds isolated from fennel and obtained in a pure state, have justified their broad (ethno)pharmacological application [1]. It has been shown that *F. vulgare* extracts possess emnenagague and galactagogue properties, antimicrobial, anti-inflammatory, antioxidant, antispasmodic, hepatoprotective and anti-platelet activities [1, 3-5], and that they could be used in treatment of the pediatric colic and some respiratory disorders [4, 5]. Fennel is considered to be a rather safe drug with rare side effects and contraindications [1]. Two fennel varieties are of pharmaceutical importance: *F. vulgare* Mill., subsp. *vulgare* var. *dulce* (Mill.) Thellung (sweet fennel) and *F. vulgare* Mill. subsp. *vulgare* var. *vulgare* (bitter fennel).

Rapid development of GC and GC/MS techniques and deconvolution software (e.g. AMDIS (Automated Mass Spectral Deconvolution and Identification System, algorithm developed by Steve Stein)) in the last 20 years have notably lowered the detection limits for a number of volatile natural compounds and even made possible the identification of GC coeluting constituents of complex mixtures [6]. This has caused a significant increase of the number of detected and successfully identified essential oil constituents (from a few tens to even several hundreds) [6]. Thus, we have decided to isolate and analyze (GC and GC/MS) essential oils and diethyl ether extracts of fennel roots and schizocarps to possibly detect and identify previously undetected/unidentified (minor) constituents. The chemical composition of the fruit volatile oils of Serbian *F. vulgare* has been reported previously [7-9]. However, the previously and currently analyzed plant materials are not of the same origin i.e. biologically uniform (they are representatives of a different genetic pool that have grown under different ecological circumstances). Therefore, the present work provides further new information on the *F. vulgare* volatile oils. An additional aim of this study was to mutually compare the chemical composition of *F. vulgare* extracts obtained from different plant organs (roots and fruits) and using different methods (hydrodistillation and solvent extraction).

2. MATERIAL AND METHODS

2.1. Plant material

Roots and schizocarps of *F. vulgare* were collected in October 2008, in the city of Niš, Serbia. Voucher specimens (collected from the same plant population, in August, 2008, during its full anthesis) were deposited in the Herbarium of the Faculty of Science and Mathematics, University of Niš, under the acquisition number 200854. Botanical identification was performed by N.R.

2.2. Essential oil isolation

Fresh *F. vulgare* roots (cut into small pieces) and crushed (in a mortar) schizocarps (three batches each) were subjected to hydrodistillation with the appropriate amount of distilled water (1-3 L) for 2-6 h (until a clear distillate was obtained) using the original Clevenger-type apparatus [10]. The obtained oils were separated by extraction with diethyl ether and dried over anhydrous magnesium sulphate. The solvent was evaporated

under a gentle stream of nitrogen at room temperature in order to exclude any loss of the essential oil and immediately analyzed. When the oil yields were determined, after the bulk of ether was removed under a stream of N_2, the residue was exposed to *vacuum* at room temperature for a short period to eliminate the solvent completely. The pure oil was then measured on an analytical balance and multiple gravimetric measurements were taken during 24 h to ensure that all of the solvent had evaporated. The yields of obtained oils (given in % (w/w) and grams) and masses of the starting plant materials (typical values), as well as the sample designations, are listed in Table 1.

2.3. Preparation of diethyl ether extracts

Fresh *F. vulgare* roots (cut into small pieces) and crushed schizocarps (three batches each) were immersed in vessels with 100-200 mL of diethyl ether, sealed and left in a dark place, at room temperature, for 5 days. The extract was gravity filtered through a small column packed with 5 g of Celite® (Merck, Darmstadt, Germany), to remove all insoluble material, and then concentrated to 10 mL at room temperature using a stream of nitrogen before GC and GC/MS analyses. The yields of dry extracts, obtained by complete evaporation of the solvent *in vacuo,* are given in Table1.

Table 1. Masses of *F. vulgare* roots and schizocarps used for the extractions, yields of the obtained *F. vulgare* volatile oils and extracts and sample designations

Plant part		Plant material mass, g[a]	Yield, % (w/w)/g[a]	Sample designation
Root	Essential oil	1000	0.2/2.05	RO
	Extract	100	0.1/0.14	RE
Schizocarp	Essential oil	500	4.5/22.68	SO
	Extract	30	3.8/1.15	SE

[a] Average values of three repetitions.

2.4. GC and GC/MS analyses

The GC/MS analyses were repeated three times for each sample using a Hewlett-Packard 6890N gas chromatograph. The gas chromatograph was equipped with a fused silica capillary column DB-5MS (5% phenylmethylsiloxane, 30 m × 0.25 mm, film thickness 0.25 μm, Agilent Technologies, USA) and coupled with a 5975B mass selective detector from the same company. The injector and interface were operated at 250 and 320 ^{o}C, respectively. The oven temperature was raised from 70 to 315 ^{o}C at a heating rate of 5 ^{o}C/min and then isothermally held for 10 min. As a carrier gas helium at 1.0 mL/min was used. The samples, 1 μL of the oil solutions in diethyl ether (1 : 100) or extracts (prepared as previously mentioned), were injected in a pulsed split mode (the flow was 1.5 mL/min for the first 0.5 min and then set to 1.0 mL/min throughout the remainder of the analysis; split ratio 40 : 1). Mass selective detector was operated at the ionization energy of 70 eV, in the 35-500 amu range with a scanning speed of 0.34 s. GC (FID) analyses were carried out under the same experimental conditions using the same column as described for the GC/MS. The percentage composition was computed from the GC peak areas without the use of correction factors. Qualitative analyses of the essential oils and

extracts constituents were based on several factors. Firstly, the comparison of the essential oils linear retention indices relative to the retention times of C_8-C_{32} n-alkanes on the DB-5MS column [11] with those reported in the literature [12]. Secondly, by comparison of their mass spectra with those of authentic standards, as well as those from Wiley 6, NIST02, MassFinder 2.3. Also, a homemade MS library with the spectra corresponding to pure substances and components of known essential oils was used, and finally, wherever possible, the identification was achieved by co-injection with an authentic sample (Table 2, see column Identification). Relative standard deviation (RSD) of repeated measurements (independent sample preparations and GC-MS) was for all substances below 1%. The only exceptions which had higher RSD were minor components such as β-phellandrene, camphor, kessane and hexadecanoic acid where RSD was 6, 2, 8 and 11%, respectively.

2.5. AMS (Average mass scan of the total ion chromatogram) profiles of analyzed samples

The average mass scans of the total ion chromatograms (AMS) of all samples (A-D) were obtained directly from the ChemStation as an average of Rt1 (2.09-2.25) to Rt2 (11.70-44.23) min and present the arithmetic average value of the abundances of each ion recorded by the mass selective detector in the given time frame, rounded to a nominal mass (35-500 amu). Large solvent peaks appearing up to a Rt of 2 min were not recorded. The duration of a single run was 59 min. After Rt2 (11.70-44.23 min) no ions corresponding to the last peak apex of the given GC chromatogram were detected and the interval between Rt2-59.00 min was not taken into account to lessen the effect of column bleed peaks.

3. RESULTS AND DISCUSSION

Analyses (GC and GC/MS) of *F. vulgare* roots and schizocarp essential oils and diethyl ether extracts enabled the identification of 89 different components, representing 98.2-100% of the total samples (Table 2). Total ion chromatograms (GC/MS) of all oils and extracts are given in Figure 1. The most abundant classes of constituents of the four analyzed samples were the phenylpropanoids (69.5-85.5%) and monoterpenoids (11.7-26.9%). The structures of the major identified components and their mass spectra are given in Figures 2 and 3. The dominant volatile metabolites of schizocarps were fenchone (13.3-18.8%) and (*E*)-anethole (66.1-69.0%). Contrary to that, terpinolene (6.2-6.5%) and dillapiole (71.4-77.5%) were the major volatiles of fennel roots. The obtained results are in general agreement with the previous studies on the volatile metabolites of *F. vulgare* [7-9, 13-21]. Nevertheless, 24 (minor) constituents (Table 2) of the analyzed samples have been for the first time identified as fennel volatiles. Moreover, this study has once again confirmed that the volatile profiles of fennel roots and fruits, being mostly indistinct from the previous reports, are not influenced by ecological and geographical factors, but only by subspecies/variety [17]. In addition, based on the literature data and herein presented results, one could assume that the plant material studied in this paper corresponds to *F. vulgare* Mill. subsp. *vulgare* var. *vulgare* (bitter fennel) [17].

Table 2. Chemical composition of *Foeniculum vulgare* Mill. roots and schizocarp essential oils (RO and SO, respectively) and diethyl ether extracts (RE and SE, respectively)

RI[a]	Compound	RO	RE	SO	SE	Class	Ident.[b]
821	3-Methylbutanoic acid		tr[c]			O	RI, MS, CoI
833	Furfural[d]	tr				O	RI, MS, CoI
902	Heptanal	tr	tr			O	RI, MS, CoI
928	α-Thujene	tr	tr		tr	M	RI, MS
936	α-Pinene	0.1	0.1	1.7	3.6	M	RI, MS, CoI
950	α-Fenchene				tr	M	RI, MS
951	Camphene			0.1	0.2	M	RI, MS, CoI
963	Benzaldehyde		tr			O	RI, MS, CoI
975	Sabinene	tr	tr	0.1	0.2	M	RI, MS
981	β-Pinene	tr	tr	0.2	0.3	M	RI, MS, CoI
990	Myrcene	0.1	0.1	0.8	1.0	M	RI, MS, CoI
991	2-Pentyl furan	tr				O	RI, MS
995	Dehydro-1,8-cineole[d]	tr				M	RI, MS
999	Mesitylene		tr			O	RI, MS, CoI
1000	Decane		tr			O	RI, MS, CoI
1003	Octanal	tr	tr			O	RI, MS, CoI
1007	α-Phellandrene	1.0	1.6	0.4	0.4	M	RI, MS
1013	*p*-Mentha-1(7),8-diene[d]	tr	tr			M	RI, MS
1019	α-Terpinene	tr	tr	tr	tr	M	RI, MS
1026	*p*-Cymene	0.6	0.7	0.1	0.1	M	RI, MS, CoI
1030	Limonene	0.3	0.4	2.7	2.5	M	RI, MS, CoI
1031	β-Phellandrene	0.1	0.1	tr	0.1	M	RI, MS
1034	1,8-Cineole			0.2	0.2	M	RI, MS, CoI
1035	(*Z*)-β-Ocimene	tr		tr	tr	M	RI, MS
1045	Phenylacetaldehyde	tr	tr			O	RI, MS, CoI
1059	γ-Terpinene	3.1	4.0	1.3	1.2	M	RI, MS
1069	*cis*-Sabinene hydrate			0.1	0.1	M	RI, MS
1091	Terpinolene	6.2	6.5			M	RI, MS
1092	*p*-Cymenene[d]	tr				M	RI, MS
1093	Fenchone			18.8	13.3	M	RI, MS, CoI
1100	Undecane	tr	tr	tr	tr	O	RI, MS, CoI
1105	3-Methylbutyl 2-methyl butanoate[d]				tr	O	RI, MS, CoI
1108	(*E*,*E*)-2,4-Heptadienal[d]			tr	tr	O	RI, MS
1114	*p*-Mentha-1,3,8-triene	tr				M	RI, MS
1117	*endo*-Fenchol	tr		tr	tr	M	RI, MS
1122	*exo*-Fenchol			tr	tr	M	RI, MS
1126	*trans*-Pinene hydrate			tr	tr	M	RI, MS
1139	*p*-Mentha-1,5,8-triene	tr				M	RI, MS
1148	Camphor			0.4	0.3	M	RI, MS, CoI
1160	(*E*)-2-Nonenal	tr	tr			O	RI, MS
1181	Terpinen-4-ol	tr		tr	tr	M	RI, MS, CoI
1187	*p*-Cymen-8-ol	tr	tr			M	RI, MS
1201	Methyl chavicol	tr		4.1	3.3	PP	RI, MS, CoI
1222	*endo*-Fenchyl acetate	0.2	0.3			M	RI, MS, CoI
1236	Hexyl 2-methylbutanoate				tr	O	RI, MS, CoI
1236	*exo*-Fenchyl acetate	tr	tr			M	RI, MS
1255	(*Z*)-Anethole			tr	0.1	PP	RI, MS
1259	*p*-Anis aldehyde				tr	PP	RI, MS

1261	(*E*)-2-Decenal[d]	tr				O	RI, MS
1288	(*E*)-Anethole	0.1	tr	69.0	66.1	PP	RI, MS, CoI
1317	*p*-Vinylguaiacol	tr				O	RI, MS
1380	α-Copaene				tr	S	RI, MS
1403	Vanillin		tr			O	RI, MS, CoI
1425	β-Caryophyllene	tr	tr			S	RI, MS, CoI
1444	β-Barbatene[d]		tr			S	RI, MS
1470	γ-Decalactone[d]	tr				O	RI, MS, CoI
1485	*trans*-Cadina-1(6),4-diene[d]			tr	0.1	S	RI, MS
1494	4-*epi*-*cis*-Dihydroagarofuran[d]	0.9	1.1			S	RI, MS
1510	β-Bisabolene	tr	tr			S	RI, MS
1525	Myristicin	4.5	2.6			PP	RI, MS, CoI
1526	δ-Cadinene				tr	S	RI, MS
1527	β-Sesquiphellandrene	tr	tr			S	RI, MS
1533	Kessane[d]	1.5	1.6			S	RI, MS
1537	Liguloxide[d]	tr				S	RI, MS
1558	Elemicin	0.2	0.2			PP	RI, MS
1577	(*E*)-2-Tridecen-1-ol[d]	tr				GL	RI, MS
1581	Propiovanillone[d]		tr			PP	RI, MS, CoI
1600	Hexadecane	tr				O	RI, MS, CoI
1636	Dill apiole	71.4	77.5			PP	RI, MS
1656	Exalatacin[d]	tr	tr			PP	RI, MS
1659	Butylphthalide[d]	tr	tr			O	RI, MS
1686	Apiole	9.3	tr			PP	RI, MS
1717	(*E*)-3-Butylidene phthalide[d]		tr			O	RI, MS
1719	Sedanenolide[d]		0.1			O	RI, MS
1735	(*Z*)-Ligustilide[d]		tr			O	RI, MS
1796	(*E*)-Ligustilide[d]		tr			O	RI, MS
1917	Methyl hexadecanoate		tr			O	RI, MS, CoI
1951	Hexadecanoic acid		0.1			O	RI, MS, CoI
2037	(*Z*)-Falcarinol	tr	0.2			O	RI, MS
2055	Bergaptene		0.2			PP	RI, MS
2145	(*Z*)-9-Octadecenoic acid				0.3	O	RI, MS
2300	Tricosane				tr	O	RI, MS, CoI
2168	Abieta-8(14),13(15)-diene[d]	tr				O	RI, MS
2188	Falcarindiol		0.5			O	RI, MS
2500	Pentacosane				tr	O	RI, MS, CoI
2700	Heptacosane				0.1	O	RI, MS, CoI
2900	Nonacosane				0.2	O	RI, MS, CoI
3080	10-Nonacosanone[e]				5.8		RI, MS
3187	Stigmast-5-en-3β-ol[d]		0.3			O	RI, MS
	Total	**99.6**	**98.2**	**100**	**99.5**		
	Monoterpenoids (M)	**11.7**	**13.8**	**26.9**	**23.5**		
	Oxygenated	0.2	0.3	19.5	13.9		
	Hydrocarbones	11.5	13.5	7.4	9.6		
	Sesquiterpenoids (S)	**2.4**	**2.7**	**tr**	**0.1**		
	Phenylpropanoids (PP)	**85.5**	**80.5**	**73.1**	**69.5**		
	Others (O)	**tr**	**1.2**	**tr**	**0.6**		

[a] Compounds listed in order of elution on DB-5MS column (RI -experimentally determined retention indices on the mention column by co-injection of a homologous series of n-alkanes C_8-C_{32}); [b] MS - constituent identified by mass spectra comparison; RI - constituent identified by retention index matching; CoI - constituent identity confirmed by GC co-injection of an authentic sample; [c] tr -trace (<0.05%); [d] Found for the first time in a *F. vulgare* sample; [e] MS (EI, 70 eV, *m/z*) 422([M^+], 3), 393(1), 365(1), 337(3), 311(12), 310(8), 295(66), 250(1), 183(11), 171(64), 155(91), 127(13), 110(24), 96(28), 95(25), 85(46), 81(21), 71(100), 59(34), 58(58), 57(81), 55(48), 43(87), 41(33).

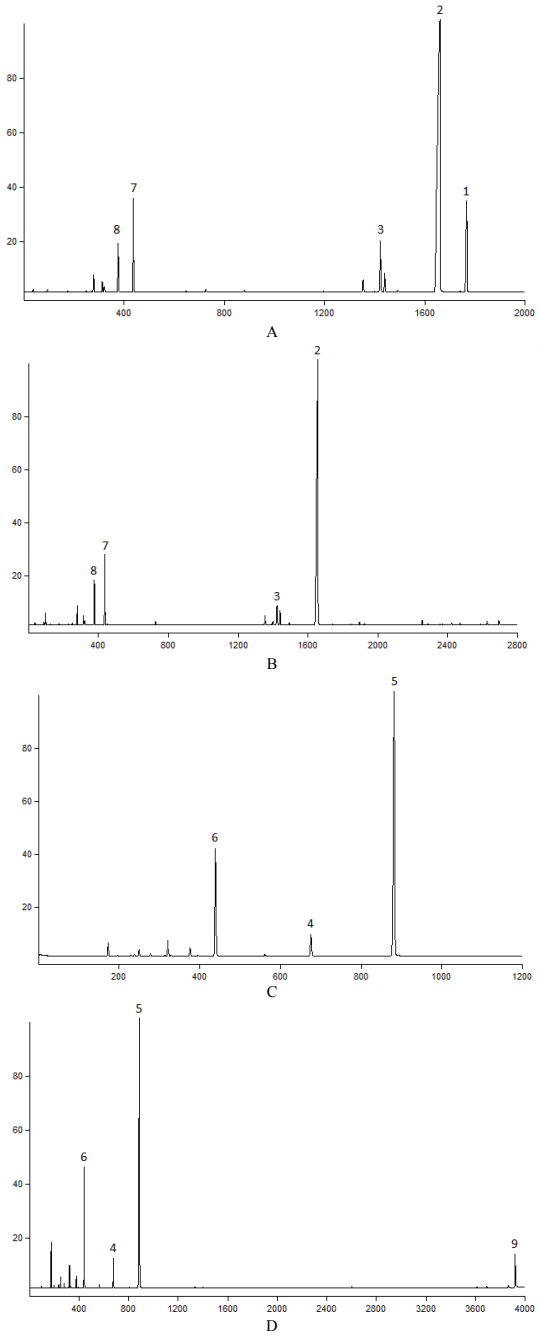

Fig. 1. Total ion chromatograms of samples RO (A), RE (B), SO (C) and SE (D) (GC/MS; x-axis: scan number, y-axis: relative abundance); for compound designations see Fig. 2.

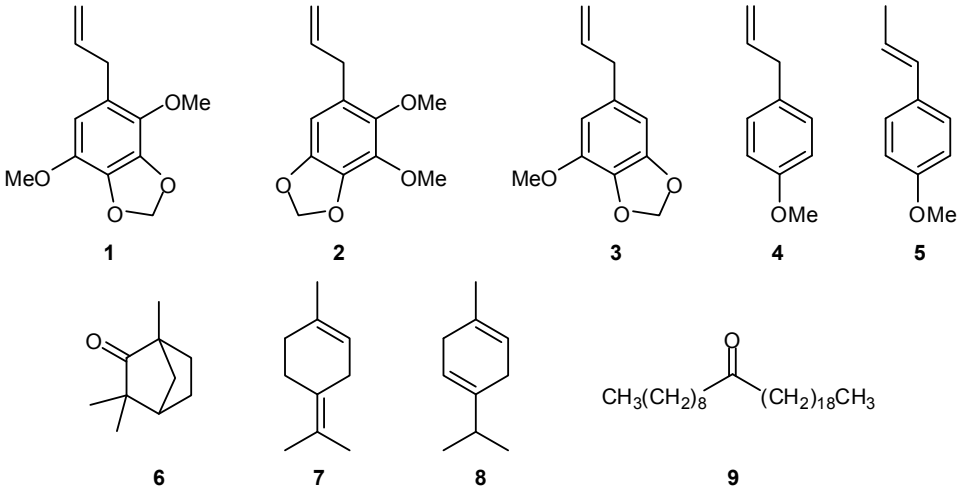

Fig. 2. The major compounds identified in the analyzed oils and extracts: **1**-apiole, **2**-dillapiole, **3**-myristicin, **4**-methyl chavicole, **5**-(*E*)-anethole, **6**-fenchone, **7**-terpinolene, **8**-γ-terpinene and **9**-10-nonacosanone.

Generally speaking, both herein analyzed oils were comparable in composition with their corresponding extracts (RO and RE; SO and SE), Table 2. The most striking difference between the root oil and the corresponding extract was the rather surprising presence of a significant relative amount of apiole in RO (9.3%), while this compound was detected only in trace amounts in RE. Two (at least) different hypotheses could be postulated to explain the mentioned difference. One possibility is that apiole was formed during hydrodistilation from some other *F. vulgare* metabolite, that was not volatile/stable under the hydrodistillation and/or GC conditions (and thus GC/MS undetectable in both the root oil and extract). However, it must be mentioned that it doesn't seem reasonable to assume that dillapiole could isomerize into apiole during the isolation of the essential oil (**1** and **2**, Figure 2). Other explanation is that the extraction of the roots (diethyl ether, ambient temperature, 5 days) was not as efficient as the hydrodistillation, and apiole remained encapsulated in the plant material. As the plant material was subjected to relatively high temperatures (around 100 °C) during the hydrodistillation, apiole could have been (readily) "released" from fennel roots and isolated together with other essential oil constituents (RO). This hypothesis could be simply tested by modifying extraction conditions (time of extraction, solvent used, ultrasound assisted extraction, etc.), however, that was outside of the scope of the current paper. One could even argue that the herein applied methodology was not completely appropriate for the extracts investigations (GC and GC/MS), since, generally speaking, some of the plant constituents, extractable from plant material with diethyl ether or other organic solvents of the similar polarity, could be non volatile under GC and GC/MS conditions (e.g. temperature of GC injector of 250 °C). Nevertheless, GC and GC/MS analyses of plant extracts allow identification of additional compounds, which cannot be steam-distilled, and thus are not present in volatile oils [13]. As an example for this, 10-nonacosanone could be used. This com-

pound, which cannot be steam-distilled (and was absent from SO), represented a significant portion of the GC and GC/MS analyzable part of fennel's fruit extract (SE, 5.8%). In fact, it is the GC and GC/MS analyses of the extract of *F. vulgare* that have enabled the first detection/identification of 10-nonacosanone as the fennel metabolite [13]. Moreover, some authors consider this compound as the chemical marker for the genera *Foeniculum*, *Anethum* and *Bupleurum*, all belonging to the Apiaceae [13]. It seems reasonable to assume that 10-nonacosanone is actually a fennel fruit wax constituent [22]. To confirm this assumption, intact schizocarps should be washed/rinsed with acetone and the obtained washings (after the solvent removal) analyzed by GC/MS. However this will be explored elsewhere.

It has been shown that in the analysis of complex volatile mixtures, the inclusion of the AMS data (average mass scan of the total ion chromatograms), alongside with the tables of identified constituents and their relative percentages, would be of great assistance. It could facilitate the creation and comparison of large data sets and provide a way for reviewers to readily verify the identification of the constituents of the complex mixtures [6, 23]. It should be noted that AMS represents the average response of the MS detector in a given timeframe. The relative abundances of the AMS *m/z* values correspond to the arithmetic mean for a given timeframe and account for both the relative abundances of ions in individual mass spectra, as well as the relative percentages of the corresponding mixture components. AMS is not an average mass spectrum of the mixture, as this would result in a loss of the information about the relative percentages of the mixture components [6, 23]. It is important to point out that AMS profiles of the essential oils, or some other GC/MS analyzable mixtures, provide additional/control data set for their multivariate statistical analyses [6, 23]. Moreover, the use of the relative abundances of the AMS *m/z* values as variables, rather than percentage compositions based on peak areas, has the potential to eliminate many of the shortcomings related to the direct application of data obtained from different research laboratories and/or instruments. Multivariate analysis of complex mixtures based on R_t values and integration of peak areas, are hampered by the very frequent event of close peak elution (or coelution), which can lead to erroneous integration results. This problem can be overcome by utilizing AMS, since it is not the elution time that is important, but rather the contributing fragmentation patterns of the different compounds [6, 23]. The AMS profiles of the herein analyzed volatile oils and extracts of *F. vulgare* roots and fruits are given in Figure 4. In fact, this is the first report of the AMS profiles of any plant originated mixture of compounds. As can be seen from Figures 3 and 4, the AMSes of the analyzed samples clearly confirm the identification of the samples' (major) constituents. For example, the most dominant peaks of the SO and SE AMS profiles were the most dominant *m/z* values (e.g. *m/z* 148, 81, 71) of the MSes of their major constituents: (*E*)-anethole, fenchone and 10-nonacosanone, Figures 3 and 4. Similarly, peaks with *m/z* 222, 177, 93 etc. (the most abundant in the MSes of dillapiole, apiole and terpinolene) were that of the highest relative amounts in the AMSes of RE and RO (Figures 3 and 4).

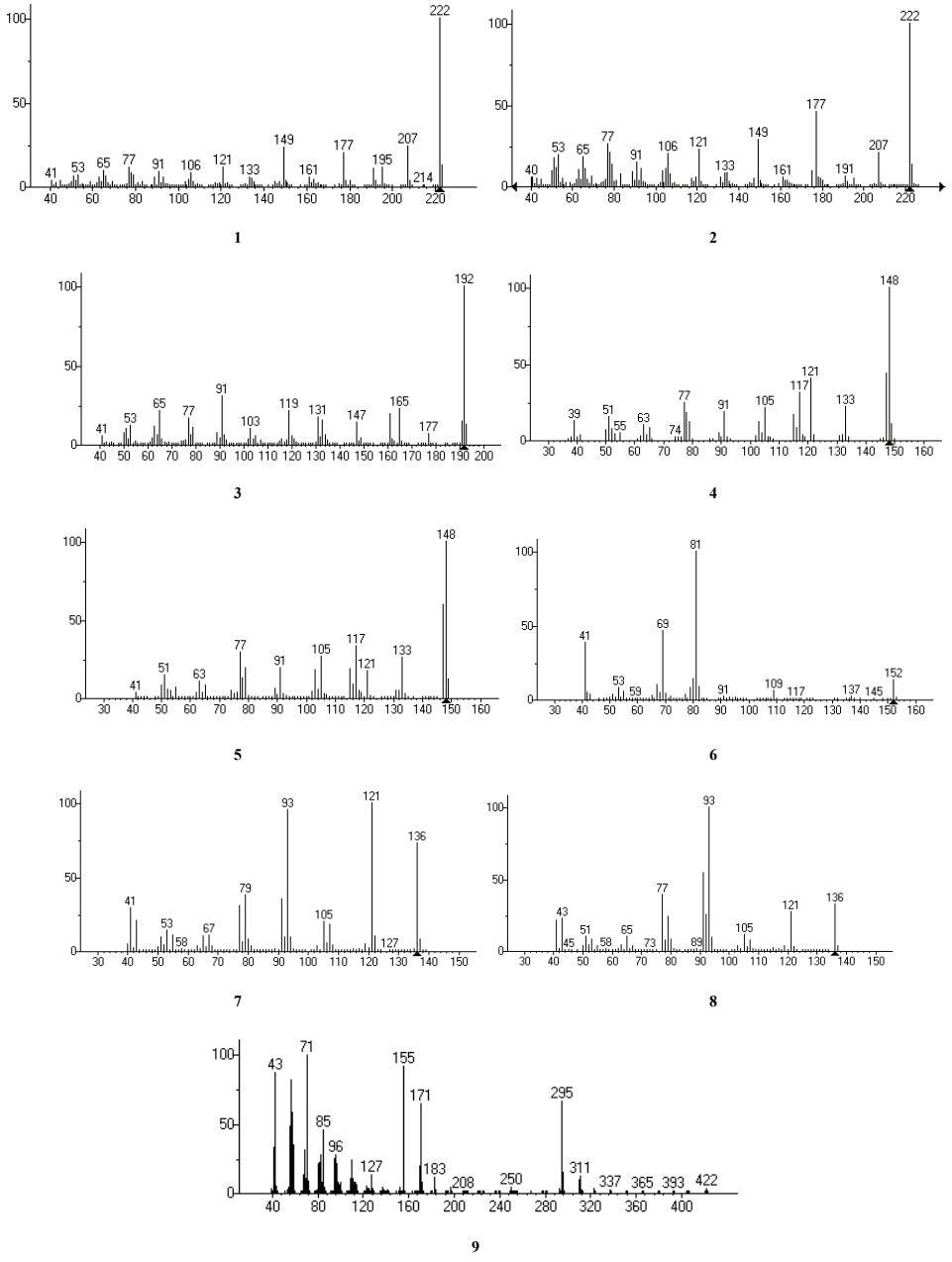

Fig. 3. Mass spectra (EI, 70 eV) of major compounds identified in the analyzed oils and extracts: **1**-apiole, **2**-dillapiole, **3**-myristicin, **4**-(*E*)-anethole, **5**-methyl chavicole, **6**-fenchone, **7**-terpinolene, **8**-γ-terpinene and **9**-10-nonacosanone.

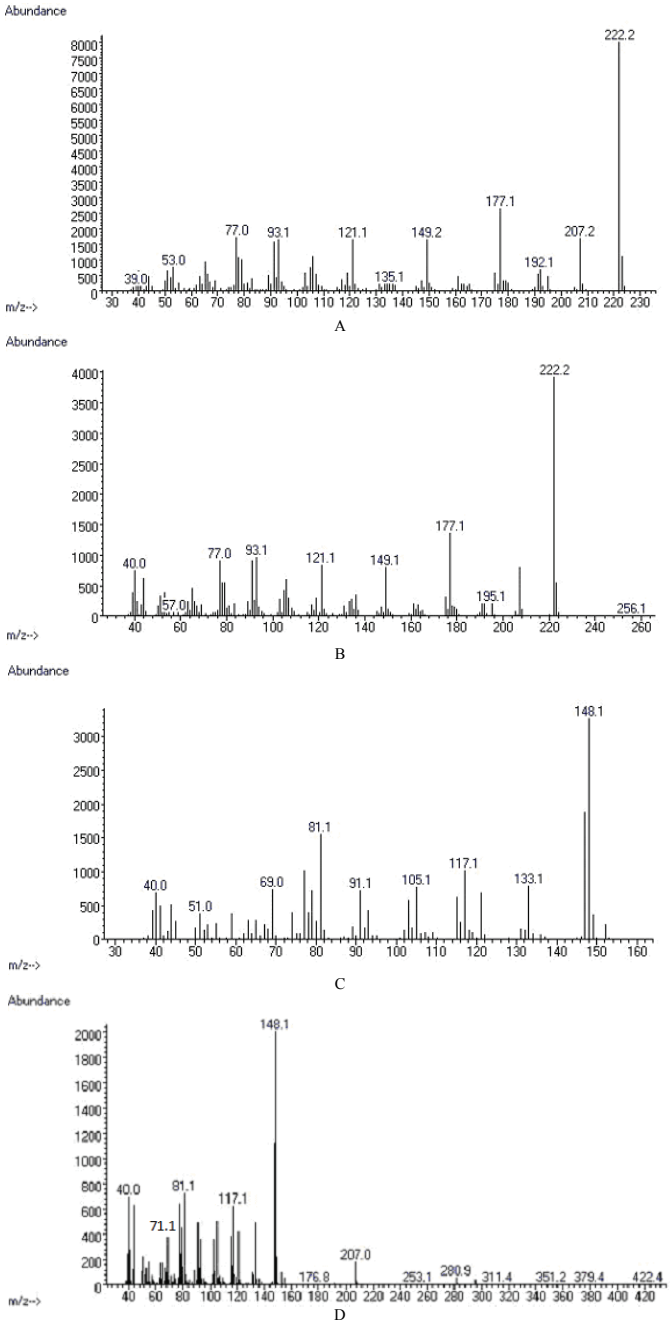

Fig. 4. AMS (average mass scan of the total ion chromatogram) profiles of samples RO (A; Rt=2.09-21.67 min), RE (B; Rt=2.25-11.70 min), SO (C; Rt=2.20-32.01 min) and SE (D; Rt=2.15-44.23 min).

To summarize, the GC and GC/MS analyses of *F. vulgare* roots and fruit essential oils and diethyl ether extracts enabled the identification of 24 components that haven't been previously reported as fennel volatiles. This once again pointed out to the importance of reinvestigation of the previously analyzed plant species, especially that of pharmacological significance.

REFERENCES

1. J. Nazaruk, *Fennel, Foeniculum vulgare Mill., Apiaceae*. In *Recent Progress in Medicinal Plants*, Vol. 28, A.S. Amani, V.K. Singh and J.N. Govil (Edts), Studium Press, LLC, Houston, Texas, 2010, 1-17.
2. M. Albert-Puleo, Fennel and anise as estrogenic agents**,** *Journal of Ethnopharmacology*, **2** (4)**,** 337-344 (1980).
3. S.N. Ostad, M. Soodi, M. Shariffzadeh, N. Khorshidi and H. Marzban, The effect of fennel essential oil on uterine contraction as a model for dysmenorrhea, pharmacology and toxicology study, *Journal of Ethnopharmacology*, **76** (3), 299-304 (2001).
4. F. Savino, F. Cresi, E. Castagno, L. Silvestro and R. Oggero, A randomized double-blind placebo-controlled trial of a standardized extract of *Matricariae recutita*, *Foeniculum vulgare* and *Melissa officinalis* (ColiMil) in the treatment of breastfed colicky infants, *Phytotheraphy Research*, **19** (4), 335-340 (2005).
5. H. Ozbek, S. Ugras, H. Dulger, I. Bayram, I. Tuncer, G. Ozturk and A. Ozturk, Hepatoprotective effect of *Foeniculum vulgare* essential oil, *Fitoterapia*, **74** (3), 317-319 (2003).
6. P. Blagojević, A new approach to the comparison of complex volatile mixtures of natural origin: a correlation between the essential oil yield and chemical composition, and percentage composition and the average mass scan of the total ion chromatogram, Ph. D. Thesis, University of Niš, Faculty of Science and Mathematics, Niš, 2010.
7. N. Mimica-Dukić, S. Kujundžić, M. Sokolović and M. Couladis, Essential oil composition and antifungal activity of *Foeniculum vulgare* Mill. obtained by different distillation conditions, *Phytotherapy Research*, **17** (4), 368-371 (2003).
8. Y.R. Naves and J. Tucakov, Presence of anetholes in the essential oils of the fennel of Yugoslavia, *Comptes rendus*, **248**, 843-845 (1959).
9. J. Tucakov, Yugoslav aromatic plants and oils, Rivista italiana essenze, profumi, piante offizinali, olii vegetali, saponi, **39**, 471-87 (1957).
10. J.P. Clevenger, Apparatus for volatile oil determination, description of new type, *American Perfumer and Essential Oil Review*, **23**, 467-503 (1928).
11. H. Van den Dool and P.D. Kratz, A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography, *Journal of Chromatography*, **11** (3), 463-471 (1963).
12. R.P. Adams, Identification of essential oil components by gas chromatography/mass spectrometry, Allured Publishing Corporation, Carol Stream, IL (2007).
13. B. Muckenstrum, D. Foechterlen, J. Reduron, P. Danton and M. Hildenbrand, Phytochemical and chemotaxonomic studies of *Foeniculum vulgare*, *Biochemical Systematics and Ecology*, **25** (4), 353-358 (1997).
14. K.H.C. Baser, T. Ozek, Kh.R. Nuriddinov, A.M. Nigmatullaev, K.Kh. Khadzimatov and Kh.N. Aripov, The Essential Oils of *Mediasia macrophylla* (Regel et Schmalh.) Pimen. and *Foeniculum vulgare* Mill. from Uzbekistan, *Journal of Essential Oil Research*, **9** (2), 249-250 (1997).
15. K. Muberra, O. Temel, M. Kurkcuoglu and K.H.C. Baser, Comparison of microwave-assisted hydrodistillation and hydrodistillation methods for the fruit essential oils of *Foeniculum vulgare*, *Journal of Essential Oil Research*, **19** (5), 426-429 (2007).
16. M. Kurcuoglu, N. Sargin and K.H.C. Baser, Composition of volatiles obtained from spices by microdistillation, *Chemistry of Natural Compounds*, **39** (4), 355-357 (2003).
17. J. Bernath and E. Nemeth, Chemical systematization of the genus *Foeniculum* Mill. based on the accumulation and qualitative differentiation of the essential oil, *Natural Product Communications*, **2** (3), 309-314 (2007).
18. J. Bernath, E. Nemeth, F. Petheo, E. Mihalik, K. Kalman and R. Franke, Regularities of the essential oil accumulation in developing fruits of fennel (*Foeniculum vulgare* Mill.) and its histological background**,** *Journal of Essential Oil Research*, **11** (4), 431-438 (1999).

19. J. Bernath, E. Nemeth, A. Kattaa and E. Hethelyi, Morphological and chemical evaluation of fennel (*Foeniculum vulgare* Mill.) populations of different origin, *Journal of Essential Oil Research*, **8** (3), 247-253 (1996).
20. F. Mojab, K. Javidnia, B. Nickavar and D. Yazdani, GC-MS Analysis of the Essential Oils of Roots and Leaves of *Foeniculum vulgare* Mill., *Journal of Essential Oil-Bearing Plants*, **10** (1), 36-40 (2007).
21. M. Gross, E. Lewinsohn, Y. Tadmor, E. Bar, N. Dudai, Y. Cohen and J. Friedman, The inheritance of volatile phenylpropenes in bitter fennel (*Foeniculum vulgare* Mill. var. *vulgare*, Apiaceae) chemotypes and their distribution within the plant, *Biochemical Systematics and Ecology*, **37** (4), 308-316 (2009).
22. N. Radulović, P. Blagojević and R. Palić, Composition of diethyl ether flower extracts of *Lonicera fragrantissima* Lindl. & Paxton (Caprifoliaceae), *Natural Product Communications*, **4** (11), 1581-1584 (2009).
23. N. Radulović, P. Blagojević and D. Skropeta, Average mass scan of the total ion chromatogram *versus* percentage chemical composition in multivariate statistical comparison of complex volatile mixtures, *Journal of Brazilian Chemical Society*, **21** (12), 2319-2326 (2010).

ISPARLJIVI SEKUNDARNI METABOLITI BILJNE VRSTE FOENICULUM VULGARE MILL. (APIACEAE)

Niko S. Radulović, Polina D. Blagojević

Ukupno 89 jedinjenja, koja su sačinjavala 98,2-100% etarskih ulja i dietil-etarskih ekstrakata korena i šizokarpa biljne vrste Foeniculum vulgare Mill. (morač), je identifikovano korišćenjem gasne hromatografije i gasne hromatografije sa masenom detekcijom (GC i GC/MS). Čak četvrtina (ukupno 24) od svih identifikovanih jedinjenja u analiziranim uzorcima nije nađena u ranije proučavanim uljima/ekstraktima morača. Najzastupljenije klase jedinjenja u svim analiziranim uzorcima su bili fenilpropanoidi (69,5-85,5%) i monoterpenoidi (11,7-26,9%). Dominantni sastojci etarskog ulja i dietil-etarskog ekstrakta šizokarpa morača su bili fenhon (13,3-18,8%) i (E)-anetol (66,1-69,0%). Nasuprot tome, uzorci dobijeni iz korena F. vulgare sadržali su značajnu količinu terpinolena (6,2-6,5%) i dilapiola (71,4-77,5%). Najznačajnije razlike u hemijskim sastavima analiziranih ulja i odgovarajućih ekstrakata se odnose na visok relativni udeo apiola u ulju korena (9,3%; u ekstraktu korena apiol je bio prisutan tek u tragovima) i značajan procenat 10-nonakozanona u ekstraktu šizokarpa (5,8%; ovaj keton nije detektovan u ulju plodova). Predložene su hipoteze za objašnjenje pomenutih razlika. U radu su dati i UMS (usrednjeni maseni sken ukupnog jonskog hromatograma) profili/"otisci prsta" analiziranih ulja i ekstrakta vrste F. vulgare.

Ključne reči: *Foeniculum vulgare Mill., Apiaceae, etarsko ulje, dietil-etarski ekstrakt, koren, šizokarpi*