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Chemical Composition of the Essential Oil from Aerial parts of *Eremostachys Macrophylla* Montbr. & Auch from Northeast of Iran

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Abstract

The essential oil obtained by hydro distillation in a Clevenger-type apparatus of the aerial parts of *Eremostachys macrophylla* Montbr & Auch, grown wild in Iran, was analyzed by GC/MS. Forty-four compounds representing 91.6% of aerial parts oil of *Eremostachys macrophylla* were identified. The main components of the oil were hexadecanoic acid (27.5%), ethyl linoleate (8.5%), 6-methyl-α-ionone (8.0%), isobutyl phthalate (5.8%), α-cadinol (4.7%) and germacrene D (4.3%). The oil was rich in nonterpenoids (56.0%) and among them; oxygenated nonterpenes (53.2%) predominated over nonterpene hydrocarbons (2.8%).

Keywords: *Eremostachys macrophylla*; Essential oil composition; Hexadecanoic acid

Introduction

The genus *Eremostachys* of the family Lamiaceae (*alt.* Labiatae) contains 15 species of perennial in Iran, and five of them are endemic [1,2]. During the past decade, seven investigations have been carried out on the chemical composition of the essential oils of the genus *Eremostachys*. These studies include the analysis of the fresh aerial parts of *Eremostachys laciniata* Bunge from Jordan [3], flowers, stems, and roots of *Eremostachys laevigata* from Iran [4], flower, leaf and stem of *Eremostachys macrophylla* and the aerial parts of *Eremostachys labiosa* from Iran [5], the aerial parts of *Eremostachys adenantha* and *Eremostachys macrophylla* from Iran [6], the aerial parts of *Eremostachys macrophylla* from Central Iran [7], the aerial parts of *Eremostachys laevigata* Bge. From Iran [8] and the aerial parts of *Eremostachys laciniata* Bge. from Iran [9].

Phytochemical investigation on a few species of *Eremostachys* revealed the presence of vicar in, a new isoflavone from *Eremostachys vicaryi* [10], eremosides A-C, new iridoid glucosides from *Eremostachys loasifolia* [11], loasifolin, a new flavonoid from *Eremostachys loasifolia* [12], a new acidic iridoid glucoside [13], furanolabdane diterpene glycosides from *Eremostachys laciniata* [14], new iridoid glucosides from *Eremostachys moluccelloides* Bunge [15] and Eremostachiin, and a new furanolabdane diterpene glycoside from *Eremostachys glabra* [16].

Our study dealt with the analysis of the essential oils of aerial parts of *Eremostachys macrophylla* grown wild in northeastern Iran. We reinvestigate the essential oil of aerial parts of the plant collected in May 2012 while in previous study plant material collected in June 2008.

Experimental

Plant material

The plant material was collected during the flowering stage in May 2012 from northern Sabzevar in Khorasan Province, Iran, at an altitude of 1580 meters. A voucher specimen (No. 218) was identified in Research Institute of Forests and Rangelands (RIFR), Tehran and it has been deposited in the herbarium of Research Center of Natural Resources, Sabzevar, Iran. The aerial parts of plant were dried in the shade (at room temperature).

Essential oil isolation

Air-dried aerial parts of *E. macrophylla* (100 g) were subjected to hydro distillation in a Clevenger-type apparatus for three hours to produce colorless oils. The yield of total volatiles was 0.18% (w/w). The oils were dried over anhydrous sodium sulfate and stored in sealed vials at 4°C before analysis.

GC/MS analysis

GC/MS analysis was carried out on a Hewlett-Packard 6890 gas chromatograph fitted with a fused silica HP-5MS capillary column (30 m × 0.25 mm; film thickness 0.32 μm). The oven temperature was programmed from 60°C to 220°C at 6°C min^{-1}. Helium was used as carrier gas at a flow rate of 1 mL min^{-1}. The chromatograph was coupled to a Hewlett-Packard 5973 mass selective detector with an ionization voltage of 70 eV.

Qualitative and quantitative analyses

Constituents of the volatile oils were identified by comparison of their retention indices relative to C9-C21 n-alkanes and of their mass spectral fragmentation pattern with those reported in the literature [17] and stored in a MS library (Wiley 275). The quantification of the components was performed on the basis of their GC peak area data from the HP-5MS column separation.

Results and Discussion

Because of the variable results obtained in previous studies and as a part of on-going work on the chemical analysis of oils obtained from the wild plants of Iran, we decided to re-investigate the oils of this specific plant The hydro distilled volatile oil from the crushed dry aerial parts of *Eremostachys macrophylla* from Sabzevar (Iran) was studied by GC/MS. The air-dried aerial parts of the plant yielded 0.18% (w/w) of a clear and colorless oil. Forty-four components, accounting for 91.6% of the compounds were identified in the aerial parts oil. (Table 1) lists formulas, percentages, and retention indices of identified compounds in the oil. As is evident from the table, the main components are hexadecanoic acid (27.5%), ethyl linoleate (8.5%), 6-methyl-α-ionone (8.0%), isobutyl phthalate (5.8%), α-cadinol (4.7%) and germacrene D (4.3%).

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No.	Compound	Formula	Percentage	RRI[b]	Class
1	Limonene	$C_{10}H_{16}$	0.1	1031	MH[c]
2	4-Terpineol	$C_{10}H_{18}O$	0.1	1179	OM[d]
3	Fenchyl acetate	$C_{12}H_{20}O_2$	0.4	1223	OM
4	Anethole	$C_{10}H_{12}O$	0.1	1285	OM
5	α-Copaene	$C_{15}H_{24}$	0.2	1364	SH[e]
6	β- Bourbonene	$C_{15}H_{24}$	0.8	1385	SH
7	β-Cubebene	$C_{15}H_{24}$	0.1	1390	SH
8	Tetradecane	$C_{14}H_{30}$	0.1	1400	NH[f]
9	β-Caryophyllene	$C_{15}H_{24}$	0.3	1418	SH
10	α-Guaiene	$C_{15}H_{24}$	0.1	1439	SH
11	Aromadendrene	$C_{15}H_{24}$	0.6	1442	SH
12	α-Humulene	$C_{15}H_{24}$	2.0	1452	SH
13	(E)-β-Farnesene	$C_{15}H_{24}$	0.1	1457	SH
14	Germacrene D	$C_{15}H_{24}$	4.3	1480	SH
15	β-Ionone	$C_{13}H_{20}O$	0.2	1488	OM
16	Bicyclogermacrene	$C_{15}H_{24}$	0.7	1500	SH
17	γ-Cadinene	$C_{15}H_{24}$	0.6	1515	SH
18	6-Methyl-α-ionone	$C_{14}H_{22}O$	8.0	1518	OM
19	δ-Cadinene	$C_{15}H_{24}$	3.5	1522	SH
20	Cadina-1,4-diene	$C_{15}H_{24}$	0.1	1533	SH
21	Germacrene D-4-ol	$C_{15}H_{26}O$	0.6	1574	OS[g]
22	Spathulenol	$C_{15}H_{24}O$	1.5	1578	OS
23	Caryophyllene oxide	$C_{15}H_{24}O$	0.5	1583	OS
24	Humulene epoxide II	$C_{15}H_{24}O$	1.7	1608	OS
25	τ-Muurolol	$C_{15}H_{26}O$	1.4	1643	OS
26	α-Cadinol	$C_{15}H_{26}O$	4.7	1656	OS
27	Tetradecanoic acid	$C_{14}H_{28}O_2$	1.8	1760	NH
28	Octadecane	$C_{18}H_{38}$	0.4	1800	NH
29	6,10,14-Trimethyl-2-Pentadecanone	$C_{18}H_{36}O$	1.7	1848	NH
30	2-Hydroxy-Cyclopentadecanone	$C_{15}H_{28}O_2$	0.4	1853	NH
31	Pentadecanoic acid	$C_{15}H_{30}O_2$	0.3	1867	NH
32	Isobutyl phthalate	$C_{16}H_{22}O_4$	5.8	1877	NH
33	Cyclohexadecane	$C_{16}H_{32}$	0.3	1883	NH
34	16-methyl-Oxacyclohexadecan-2-one	$C_{16}H_{30}O_2$	0.3	1943	NH
35	Sandaracopimara-8(14),15-diene	$C_{20}H_{32}$	2.5	1969	DH[h]
36	di-Butylphthalate	$C_{16}H_{22}O_4$	0.9	1973	NH
37	Hexadecanoic acid	$C_{16}H_{32}O_2$	27.5	1977	NH
38	Eicosane	$C_{20}H_{42}$	2.0	2000	NH
39	Heptadecanoic acid	$C_{17}H_{34}O_2$	0.4	2065	NH
40	Methyl linoleate	$C_{19}H_{34}O_2$	0.6	2084	NH
41	Phytol	$C_{20}H_{40}O$	0.4	2111	OD[i]
42	(Z,Z)-9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	2.7	2136	NH
43	Ethyl linoleate	$C_{20}H_{36}O_2$	8.5	2164	NH
44	Octadecanoic acid	$C_{18}H_{36}O_2$	2.3	2172	NH
	Total identified		91.6		

[a]The compounds have been arranged according to their retention indices on an HP-5 MS capillary column
[b]Kovatz retention indices given in the literature
[c]Monoterpene hydrocarbons
[d]Oxygenated monoterpene
[e]Sesquiterpene hydrocarbons
[f]Nonterpene hydrocarbons
[g]Oxygenated sesquiterpenes
[h]Diterpene hydrocarbons
[i]Oxygenated diterpenes

Table 1: Constituents of the essential oils from aerial parts of *Eremostachys macrophylla* obtained by hydrodistillation[a]

In this study, GC/MS analysis method revealed monoterpenoid hydrocarbon (MH), oxygenated monoterpenes (OM), sesquiterpenoid hydrocarbons (SH), oxygenated sesquiterpenes (OS), nonterpenoid hydrocarbons (NH), diterpene hydrocarbon (DH) and oxygenated diterpene (OD) in the oil from the aerial parts of *E. macrophylla*. One monoterpene hydrocarbon (0.1%), five oxygenated monoterpenes (8.8%), thirteen sesquiterpene hydrocarbons (13.4%), six oxygenated sesquiterpenes (10.4%), seventeen nonterpene hydrocarbons (56.0%), one diterpene hydrocarbon (2.5%) and one oxygenated diterpene (0.4%) were detected in this oil. These data lead to a rank order of constituent groups: NH>SH>OS>OM>DH>OD>MH for the aerial parts oil. The main components in this oil were hexadecanoic acid (27.5%), ethyl linoleate (8.5%), 6-methyl-α-ionone (8.0%), isobutyl phthalate (5.8%), α-cadinol (4.7%) and germacrene D (4.3%).

Thus the oil consisted mainly of nonterpenes and relatively small fractions of terpenoids. Also, oxygenated nonoterpenes (53.2%) predominated over nonterpene hydrocarbons (2.8%).

However, a previous study [6] on volatile oil from aerial parts of *E. macrophylla* found, among the thirty-five identified compounds comprising 92.9% of the oil, that the major components were spathulenol (23.4%), hexadecanoic acid (13.5%) and caryophyllene oxide (9.3%). On the other hand, another study [7] of oil from the aerial parts of this plant reported that, of the sixteen identified compounds comprising 96.4% of the oil, the major ones were germacrene-D (47.1%), germacrene-B (17.8%), γ-elemene (9.1%), myrcene (6.7%), β-elemene (2.7%), and β-phellandrene (2.6%). In addition, we also reported analysis of the essential oils from flowers, leaves and stems of *E.s macrophylla* [5]. However this specimen had been collected at different place, time and altitude from that in the current study. The major compounds in the flower oil of *E. macrophylla* were 1,8-cineol (19.0 %) and germacrene D-4-ol (10.6 %), whereas the leaf oil contained α-pinene (30.0 %), 1,10-di-epi cubenol (22.7 %), elemol (13.3 %) and bornyl acetate (11.0 %). The stem oil of the plant consisted mainly of 1,10-di-epi cubenol (34.4%) and elemol (24.0 %) [5].

It is evident from the above data, that there are significant differences in the results of the current study with previous ones [5-7] for the aerial parts of *E. macrophylla*. There may be differences related to environmental conditions such as climate, altitude, collection time, ground composition of the sampling area and different growth stages such as pre-flowering, fresh flowering and air-dried-flowering stages.

Conclusion

The chemical composition of the essential oil of aerial parts from *Eremostachys macrophylla* growing in Sabzevar was investigated. This study showed considerable amounts of hexadecanoic acid (27.5%), ethyl linoleate (8.5%), 6-methyl-α-ionone (8.0%). These major constituents were different from those seen in previous studies on the same species [5-7]. These results demonstrated that the chemical composition of the essential oil of the same species can change depending on a variety of conditions, including climate, time of collection, and the ground composition of the sampling area, in addition to the growth stages of the plant.

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