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Constituent composition of *Chenopodium botrys* essential oil

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The volatile oils of *Chenopodium botrys* L. collected in six locations throughout Southern Bulgaria were obtained by hydrodistillation and analyzed by GC/FID and GC/MS. In the essential oils from the aerial parts of *C. botrys* fifty three components were identified. The chemical composition of investigated oils differed only quantitatively. Most of the constituents were oxygenated sesquiterpenes (69.08%-84.83%). The dominant components in the oils were elemol acetate (14.01%-26.32%), elemol (10.89%-18.15%), *α*-eudesmol (7.49%-17.84%), juniper camphor (3.58%-11.49%), *α*-eudesmol acetate (5.28%-6.90%), *α*-chenopodiol (4.04%-6.40%). For first time in the essential oils of *C. botrys* *γ*-costol was identified.

Key words: *Chenopodium botrys, essential oil, elemol, elemol acetate, γ-costol*

INTRODUCTION

Chenopodium botrys L. is widespread throughout Europe, West Asia and North America. The plant has been used traditionally for medicinal purposes. The essential oils isolated from aerial parts of *C. botrys* collected from Southern Serbia and Greece exhibited significant bactericidal and fungicidal activity against selected strains of microorganisms, viz. *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Aspergillus niger, Candida albicans, Sarcina lutea, Klebsiella pneumoniae, Salmonella enteridis and Shigella flexneri* [1, 2]. The *C. botrys* essential oil from Saudi Arabia possessed antimicrobial activity [3]. The alcoholic and aqueous extracts of *C. botrys* from Iran were found to have in-vitro giardicidal effect against *Giardia lamblia* cysts [4]. The oil from the aerial part of *C. botrys* collected from suburb of Kashan, Iran exhibited strong antimicrobial activity against *Staphylococcus saprophyticus* followed by *Klebsiella pneumoniae*, *Bacillus cereus*, *Staphylococcus epidermidis*, *Streptococcus mutans*, *Listeria monocytogenes* and *Salmonella typhimurium*. The oil had slight effect on *Candida albicans* and showed inhibitory effect on *Aspergillus* species and *Bacillus subtilis* [5].

The chemical composition of the essential oils of *C. botrys* varied in amount and composition according to the environmental conditions. *C. botrys* of different origins yielded 0.08-2% essential oil [6]. According to several studies, the essential oils were rich in monoterpenes [7, 8] and sesquiterpenes [2, 3, 5, 9, 10]. De Pascuale *et al* reported the isolation of sesquiterpenes of eudesmane and guaiane type from methanol extracts of *C. botrys* [11].

The sesquiterpenes *α*- and *β*-eudesmol were found to be the major compounds in essential oil from Saudi Arabia [3]. The main components of *C. botrys* oil from North America were *α*- and *β*-chenopodiol (36%), eudesma-3,11-dien-6*α*-ol (9.4%), botrydiol (9.0%), elemol (6.5%), elemol acetate (5.5%), *γ*-eudesmol (5.4%), and *α*- and *β*-eudesmol (3.7%); guaia-3,9-dien-11-ol (7.4%) [10]. In the oil from Greece the main components were elemol acetate (16.3%), elemol (14.1%), botrydiol (11.1%), *α*-chenopodiol (9.5%) and selina-3,11-dien-6*α*-ol (6.1 %) [2]. Juniper camphor (16.5% and 25.7%), elemol (14.3% and 13.4%) and *α*-cadinol (8.2% and 11.6%), were the main compounds in the oils from two different locations in Iran [9]. *C. botrys* essential oil from Kashan, Iran contained 2,3-dehydro-4-oxo-*β*-lonone (22.4%), (+)-7-*epi*-amiteol (11.5%), elemol (7.4%), *α*-cadinol (7.0%) and *tau*-cadinol (7.0%) [5]. Major components of the essential oil of *C. botrys* collected in North of Iran were *γ*-terpineol (52.8%), p-cymene (19.0%) and *iso*-ascaridole (7.0%) [6]. Ascaridole (7.5% and 40%) was identified in *C. botrys* oils collected in Spain and Slovakia, respectively [8]. In Israel (Negev Desert) *α*-terpinene (21.4%), p-cymene (15.2%), *E*-caryophyllene (6.5%), limonene (6.1%) were identified as major components [8]. 2-(4*α*.8-dimethyl-1.2.3.4.4*α*.5.6.7-octahydro-naphthalen-2-yl)-prop-2-en-l-ol (27.9%) was the main compound in the essential oil of *C. botrys*, growing in Turkey [12].

In the present paper, the chemical composition

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of the oils obtained from the aerial parts of *C. botrys* harvested in six locations throughout Southern Bulgaria was examined.

EXPERIMENTAL

Plant material

The specimens of *C. botrys* were collected in six locations of Southern Bulgaria: Balkan Mountain (Shipka town), Plovdiv, Kaloyanovo, Rogosh, Ustina and Rodopa Mountain (Krichim town). The plants were collected in August, consecutively in 2012 and 2013 years, at full flowering stage. The plant material was identified by means of voucher specimens (05269 and 05271) by Assoc. Prof. Koicho Koev PhD (Faculty of Biology, Plovdiv University, Bulgaria). The voucher specimens were deposited in the herbarium of Agricultural University - Plovdiv. The aerial parts of the plants were dried at room temperature in the absence of direct sunlight. The dry material was further ground to powder and was packed into multilayer paper bags, and stored in a dark room at ambient temperature.

Isolation of essential oils

The dry powdered plant material (100 g) was subjected to hydrodistillation through a Clevenger-type apparatus for 4 h. The oil was dried on anhydrous Na_2SO_4 and kept in a cold and dark place until use.

GC –FID analysis

The Agilent 7890 GC-FID (Agilent Technologies, CA, USA) was equipped with capillary column HP-5 Agilent Technologies, CA, USA, 30 m x 0.32 mm i.d., film thickness 0.25 μm was used. The injector and detector temperatures were set at 200°C and 300°C, respectively. The column was set at 50°C for 5 min and the temperature was increased with 5°C /min to 180°C, from 180°C with 7°C /min to 230°C. The carrier gas was nitrogen at a flow rate of 1 ml/min. The split ratio was 100:1 and the injection volume was 0.2 μl.

The quantitative analysis (expressed in percent) was calculated by peak area normalization.

GC/MS analysis

GC/MS analysis was carried out using Agilent 7890 GC system combined with Agilent 5975 Inert MSD detector (quadrupole) with electron impact ionization (70 eV). A HP-5-MS column (30 m x 0.25 mm i.d., film thickness 0.25 μm) Agilent Technologies, CA, USA was used. The column was set at the same temperature program used in the GC-FID analysis. Scan time and mass range were 2 s and 50–550 m/z, respectively. The carrier gas was helium at a flow rate 1 ml/min. Samples were injected with a split ratio of 70:1 and 100:1 and the injection volume was 0.2 μl.

Identification of the compounds

Identification of the volatile constituents was established by comparison of recorded mass spectra with those of a computer library NIST 08 database (National Institute of Standardization and Technology, Gaithersburg, MD, USA) and with those of authentic compounds. Peaks identity was confirmed by referring to Kovats index data generated from a series of alkanes (relative to C_6–C_{22} alkanes) and with the data published in the literature [13].

RESULTS AND DISCUSSION

The essential oils obtained from aerial parts of *C. botrys* were analyzed by GC/FID and GC/MS. Representative chromatographic profile of *C. botrys* essential oils is shown on Figure 1. The GC conditions led to a good separation of all components of the oils (Figure 1).

The chemical composition of investigated oils differed only quantitatively. The yield of oils of *C. botrys* from six different locations of Southern Bulgaria and two consecutive harvests ranged from 0.33% to 1.3% (v/w on dry weight basis) (Table 1).

These data point to lower yield, comparing to the yield of essential oils obtained in Greece (1.79%) [2] and Saudi Arabia (2%) [3]. In the essential oils from *C. botrys* fifty-three compounds were identified representing 86.24% to 100% of the total oil. Among these, 69.08%-84.83% belong to the class of oxygenated sesquiterpenes. The high content of oxygenated sesquiterpenes is consistent with the data on the oil composition from North America, Greece and Iran [2, 9, 10].

The main components (%) of the *C. botrys* oils from Southern Bulgaria were elemol acetate (14.01-26.32), elemol (10.89-18.15), *α*-eudesmol (7.49-17.84), juniper camphor (3,58-11,49), *α*-eudesmol acetate (5.28-6.90) and *α*-chenopodiol (4.04-6.40) (Table 1, Figure 2).

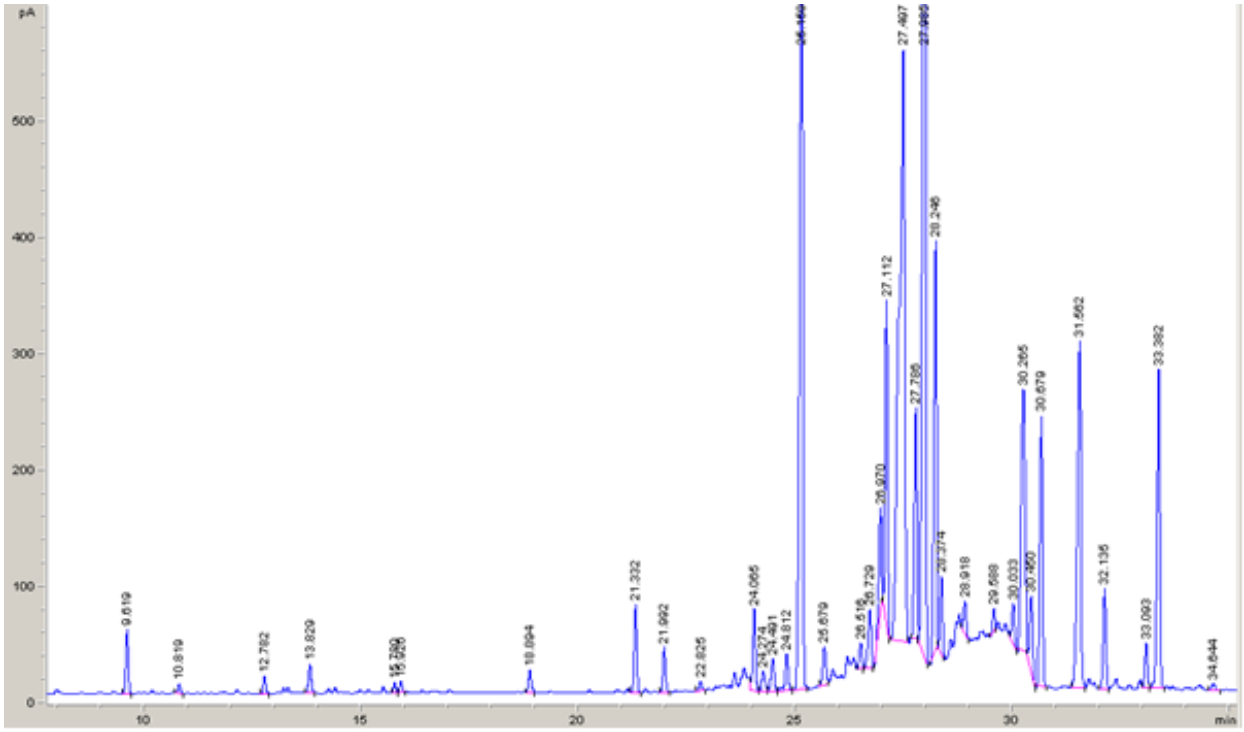

Figure 1. Representative chromatographic profile of essential oils of *C. botrys.*

Table 1. Chemical composition of essential oils of *C. botrys*. Total number of components, content obtained by normalization [%], yield [% v/w]. RI is retention index relative to C_6 – C_{22} n-alkanes on the HP-5MS column, MS-comparison of mass spectra with those listed in the NIST 08, tr = traces (< 0.1%).

Compounds	RI	*C. botrys* from different locations of Bulgaria, %					
		Sh-12	K-12	Kr-12	Pl-13	U-13	R-13
Bornylene	893	tr	tr	tr	tr	tr	tr
α-Pinene	908	tr	tr	tr	tr	tr	tr
β-Pinene	925	tr	tr	tr	tr	tr	tr
α-Fenchene	939	tr	tr	tr	tr	tr	tr
Camphene	941	tr	tr	tr	tr	tr	0.64
2,3-Dehydro-1,8-cineole	988	tr	tr	tr	tr	tr	tr
Myrcene	990	0.83	2.51	8.49	0.14	0.52	7.89
α-Limonene	1030	0.11	0.34	tr	tr	tr	0.83
γ-Terpinene	1062	tr	tr	tr	tr	tr	tr
(Z)-Sabinene hydrate	1072	tr	tr	tr	tr	tr	tr
Fenchone	1091	0.23	0.42	tr	0.15	0.23	1.12
exo-Fenchol	1117	tr	tr	tr	tr	tr	tr
cis-β-Terpineol	1126	0.43	0.77	tr	0.47	0.60	1.72
Terpinol hydrate	1139	tr	tr	tr	tr	tr	tr
p-Menth-2-en-1-ol	1145	tr	tr	tr	tr	tr	tr
trans-dehydro-α-Terpineol	1148	tr	tr	tr	tr	tr	tr
Borneol	1169	tr	tr	tr	tr	tr	tr
Terpinen-4-ol	1180	0.12	0.20	tr	tr	tr	tr
3,9-Epoxy-1-p-menthene	1187	tr	tr	tr	tr	tr	tr
α-Terpineol	1193	0.14	0.21	tr	tr	0.15	tr
cis-Piperitol	1198	tr	tr	tr	tr	tr	tr

Table 1. *Continued.*

Compounds	RI	*C. botrys* from different locations of Bulgaria, %					
		Sh-12	**K-12**	**Kr-12**	**Pl-13**	**U-13**	**R-13**
p-Menth-1-en-9-ol	1311	0.33	0.43	7.11	0.15	0.19	tr
α-Copaene	1380	tr	tr	tr	tr	tr	tr
β-Elemene	1395	1.20	1.16	5.09	0.88	1.34	1.39
α-Gurjunene	1414	tr	tr	tr	tr	tr	tr
β-Caryophyllene	1425	0.63	1.37	4.70	0.78	0.98	1.27
α-Humulene	1460	0.14	0.31	tr	0.18	0.20	tr
Z-Caryophyllene	1475	tr	tr	tr	tr	tr	tr
Germacrene D	1488	tr	tr	tr	tr	tr	tr
β-Selinene	1494	0.21	0.23	tr	0.14	0.14	tr
α-Selinene	1503	0.26	0.31	tr	0.22	0.28	tr
α-Muurolene	1508	tr	tr	tr	tr	tr	tr
Germacrene A	1515	1.02	2.59	5.54	2.17	2.47	2.82
γ-Cadinene	1521	0.32	0.41	tr	0.28	0.40	tr
δ-Cadinene	1530	0.52	0.59	tr	0.61	0.77	0.74
α-Copaen-11-ol	1545	0.51	0.42	tr	0.26	0.32	tr
α-Calacorene	1551	tr	tr	tr	tr	tr	tr
Elemol	1560	13.53	10.89	18.15	12.95	14.80	15.15
Caryophylene oxide	1583	0.60	1.00	tr	0.97	0.88	1.07
γ-Eudesmol	1644	1.76	1.30	tr	1.00	1.12	1.00
γ-Costol	1653	4.22	4.35	tr	4.76	2.94	2.56
α-Eudesmol	1671	17.56	17.84	7.49	14.92	13.10	12.95
Elemol acetate	1693	16.77	14.01	26.32	18.95	19.25	14.42
Juniper camphor	1713	6.05	3.92	11.49	3.58	5.27	4.72
Guaiol acetate	1735	0.45	0.87	tr	0.76	1.49	1.16
Hinesol acetate	1787	0.57	0.65	tr	0.70	0.68	0.60
α-Eudesmol acetate	1799	5.58	6.07	5.63	6.90	5.70	5.28
β-Chenopodiol	1830	4.07	2.65	tr	3.09	3.52	2.45
α-Chenopodiol	1876	6.34	4.04	tr	6.00	6.40	4.73
β-Chenopodiol-6-acetate	1904	1.43	1.47	tr	1.40	1.25	1.05
Acetoxyeudesman-4-*α*-ol-11	1957	0.61	0.60	tr	0.77	0.67	0.66
α-Chenopodiol-6-acetate	1977	4.78	4.31	tr	5.72	4.51	3.89
n-Heneicosane	2087	tr	tr	tr	tr	tr	tr
Total compounds		53	53	53	53	53	53
Yield, % (v/w)		0.6	0.6	0.33	0.68	1	1.3
Total identified, %		91.32	86.24	100.00	88.9	90.17	90.11
Monoterpenes, %		0.94	2.85	8.49	0.14	0.52	9.36
Oxygenated monoterpenes,%		1.25	2.03	7.11	0.77	1.17	2.84
Sesquiterpenes, %		4.3	6.97	15.33	5.26	6.58	6.22
Oxygenated sesquiterpenes, %		84.83	74.39	69.08	82.73	81.9	71.69

Sh-Shipka, K-Kaloyanovo, Kr-Krichim, Pl-Plovdiv, U-Ustina, R-Rogosh
12 and 13 - Harvest in two consecutive years 2012 and 2013, respectively

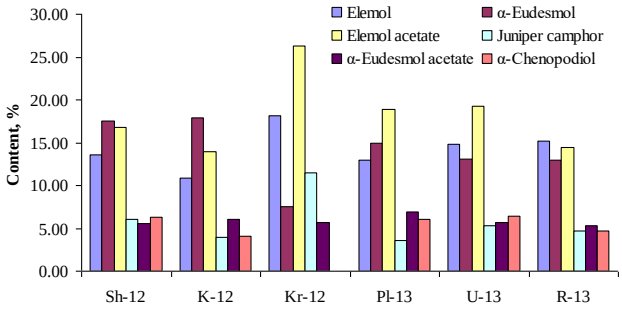

Figure 2. Content (%) of the main components in *C. botrys* essential oils

This composition of oils is close to the data reported for the essential oil from Greece [2]. Elemol acetate was not found in the essential oil from Iran [5,9]. Thus, there are significant differences in the qualitative composition of the essential oil of *C. botrys* from different geographical locations. The essential oil from Greece and North America contained botridiol [2,10], from Iran 2,3-dehydro-4-oxo-*β*-lonone and (+)-7-*epi*-amiteol [5]. These compounds were not found in the investigated essential oils from Southern Bulgaria. In all oils γ-costol was identified, which has not been reported by other authors. The components γ-costol, *α*- and *β*-chenopodiol, *α*- and *β*-chenopodiol-6-acetate in the oil from Krichim (Kr-12) were in trace amounts (Table 1). Nevertheless, the content of oxygenated sesquiterpenes in this oil is lower than 70%, the content of elemol acetate (26.32%) and elemol (18.15%) is the highest, comparing to others (Figure 2). Carroll *et al.* tested oil rich in elemol against mites *Ixodes scapularis* and *Amblyomma americanum* and have found that about 2-4 hours oil repel mites over 50% [14].

CONCLUSION

The chemical composition of essential oils of *C. botrys* from six different locations of Southern Bulgaria in two consecutive harvests differed only quantitatively. The oil is rich in elemol and elemol acetate. For first time in the essential oil of *C. botrys* γ-costol was identified.

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СЪСТАВ НА ЕТЕРИЧНОТО МАСЛО ОТ *Chenopodium botrys*

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(Резюме)

Етеричното масло от *Chenopodium botrys L.*, събиран от шест района на Южна България, беше получено чрез хидродестилация и анализирано с ГХ/ПИД и ГХ/МС. В етеричните масла от наземната част на *C. botrys* бяха идентифицирани петдесет и три съединения. Химичният състав на изследваните масла се различава само количествено. В състава на маслото пробладават сесквитерпеноиди (69.08%-84.83%). Основните компоненти на етеричното масло от *C. botrys* са елемолацетат (14.01%-26.32%), елемол (10.89%-18.15%), α-еудезмол (7.49%-17.84%), хвойнов камфор (3.58%-11.49%), α-еудезмол ацетат (5.28%-6.90%), α-хеноподиол (4.04%-6.40%). В етеричното масло от *C. botrys* за първи път беше идентифициран γ-костол.

Ключови думи: *Chenopodium botrys, етерично масло, елемол, елемол ацетат, γ-костол*