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Short Communication

Chemical composition of *Chenopodium botrys* L. (Chenopodiaceae) essential oil

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Abstract

Chemical composition of essential oil isolated from aerial parts of *Chenopodium botrys* L. (Chenopodiaceae) collected from five different locations in the Republic of Macedonia was analysed by GC/FID/MS. Seventy five compounds were identified representing 90.02-91.24% of the oil. The analysis has shown that the oils were rich in sesquiterpene components (83.18-87.54%) comprising elemol acetat (9.88%-21.98%), seline-11-en-4α-ol (9.81%-13.5%), selina-3,11-dien-6α-ol (6.42%-9.71%) and elemol (5.57%-9.49%) as major oxygen containing sesquiterpenes, followed by lower content of α-eudesmol acetat (3.24%-4.11%), α-chenopodiol (2.42%-5.43%), botrydiol (1.87-5.73%) and α-chenopodiol-6-acetat (1.9%-4.73%).

Keywords: *Chenopodium botrys*, essential oil composition, gas-chromatography/mass spectrometry (GC/MS), sesquiterpenes

Introduction

Chenopodiaceae is a large family consisting of approximately 102 genera and 1400 species (Kokanova-Nedialkova et al., 2009). The typical genus *Chenopodium* comprises numerous species of perennial and annual plants known as goosefoots, which occur anywhere in the world. According to Fuentes-Bazan et al. (2012) the species of *Chenopodium* could grow as herbaceous plant or as shrubs and small trees and they are mainly non-aromatic but could be fetid. Only few species produce essential oil (glandular goosefoots) usually with characteristic chemical composition. Thus, for South America (Argentina) *C. ambrosioides* L., *C. multifidum* L., *C. pumilio* R. Br., and few other species were claimed as aromatic (Bonzani et al., 2003). In Europe, the most interesting is *C. botrys* L. (syn. *Dysphania botrys* (L.) Mosyakin and Clemants, known as Jerusalem Oak Goosefoot also called Feathered Geranium, native to the Mediterranean region. As native or introduced plant it also grows in the territory of Asia, India, Himalayas, Northern Europe, Turkey, Cyprus, Africa, Australia and North and South America (Seidemann, 2005). *Chenopodium* species have been used in folk medicine worldwide for treatment of different ailments. It is well known that *C. album* improves the appetite and act as anthelmintic, laxative, diuretic and tonic. In South America *C. ambrosioides* is used against intestinal parasites from immemorial time. The plant is also known as carminative, diaphoretic and emmenagogue and as a remedy aginst cough, pulmonary obstruction and amenorrhea (Yadav et al., 2007). In Europe, *C. botrys* has been used for treatment of catarrh and humoral asthma and is known as a good substitute for *C. ambrosioides* (Yadav et al., 2007).

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C. botrys possesses glandular trichomes that produce essential oil with intense characteristic odour reminiscent to the frankincense scent. Essential oil of this plant has been studied and presence of some oxygen-containing sesquiterpenes was correlated with pronounced antibacterial and antifungal activity (Kokanova-Nedialkova et al., 2009; Yadav et al., 2007; Tzakou et al., 2007). Iranian researchers in essential oil of *C. botrys* showed presence of 2,3-dehydro-4-oxo-β-ionon, (+)-7-*epi*-amiteol, elemol, α-cadinol and *tau*-cadinol as main components (Mahboubi et al., 2011). In the essential oil of Greek *C. botrys*, Tzakou et al. (2007) have identified the sesquiterpenes: elemol acetate, elemol, botrydiol, α-chenopodiol, β-eudesmol and selina-3, 11-dien-6α-ol as major components. For *C. botrys* from Saudi Arabia, El-Sayed et al. (1989) showed presence of oxygen-containing sesquiterpenes, primarily α and β-eudesmol while Chalabian et al. (2006) identified α-eudesmol, *epi*-α-muurolol and cubenol as major constituents.

In the flora of the Republic of Macedonia (RM), 15 species of the genus *Chenopodium* occur including *C. botrys*, *C. multifidum* and *C. ambrosioides* as aromatic plants and 12 other species without glands (non-aromatic) such as *C. bonus-henricus*, *C. hybridum*, *C. glaucum*, *C. murale*, *C. album* etc. (Miceveski, 1995). Dried herbal parts of *C. botrys* are used from local people for preparing infusions or liquid extracts with diuretic, antispasmodic, carminative and antidiarrheal properties (Maksimovic et al., 2005). The herb of the plant has not been issue of chemical characterisation so far. Therefore, the aim of this study is determination of the chemical composition of the essential oil of wild samples of *C. botrys* collected from different localities of the Republic of Macedonia.

Materials and methods

Plant material

The aerial flowering parts of *C. botrys* were collected in the period from July to September in 2013 at five localities in RM: Kozuf, Pretor, Strumica, Zletovo and Radovish. Botanical identification of the species was performed at the Department of Pharmaceutical Botany, Faculty of Pharmacy, University Ss. Cyril and Methodius, Skopje, RM. Voucher specimens (CB-K-1/13; CB-P-1/13, CB-S-1/13, CB-Z-1/13 and CB-R-1/13 for samples from Kozuf, Pretor, Strumica, Zletovo and Radovish, respectively) were deposited at the herbarium of the Faculty of Pharmacy, University Ss. Cyril and Methodius, Skopje, RM.

Collected plant material was air-dried and preserved in paper bags until analysis was performed, when it was minced and homogenised appropriately.

Isolation of the essential oil

The essential oil was isolated with steam distillation using all-glass Clevenger type apparatus according to the European pharmacopoeia method (Ph. Eur. 7; Method 2.8.12.). Briefly, 20 g of dry plant material was measured and transferred in a balloon of Clevenger distillation apparatus, in which 500 ml of distilled water and 0.5 ml of xylene were added and distillized for 2 hours. The obtained oil in xylene was treated with anhydrous sodium sulfate in order to be purified and dried.

GC/FID/MS analysis of essential oil

Essential oil samples were analysed on Agilent 7890A Gas Chromatography system equipped with FID detector and Agilent 5975C Mass Quadrupole detector as well as capillary flow technology which enables simultaneous analysis of the samples on both detectors. For that purpose, HP-5ms capillary column (30 m x 0.25 mm, film thickness 0.25 μm) was used. Operating conditions were as follows: the oven was heated to 60 °C with a gradual increasement in temperature of 3°C/min to 240°C, which was maintained 1 min and then at rate of 10 °C/min the temperature was rised to 280 °C and held 1 min; helium as carrier gas at a flow rate of 1ml/min; injector temperature 220 °C and that of the FID 270 °C. 1μl of each sample was injected at split ratio 1:1. The mass spectrometry conditions were: ionization voltage, 70 eV, ion source temperature 230 °C, transfer line temperature 280 °C and mass range from 50 - 500 Da. The MS was operated in scan mode. For GC/FID/MS analysis, the essential oil was dissolved in xylene to obtain 1 μl/ml oil solution.

Identification of the components present in essential oils was made by comparison of obtained mass spectra with those from Nist, Wiley and Adams mass spectra libraries, using AMDIS (Automated Mass Spectral Deconvolution and Identification System) as well as by literature and estimated Kovat′s (retention) indices that were determined using mixture of homologous series of normal alkanes from C_9 to C_{25} in hexane, under the same above mentioned conditions.

The percentage ratio of essential oils components was computed by the normalisation method of the GC/FID peak areas without any correction factors.

Results and discussion

The results of the analysis of the chemical composition of essential oils isolated from samples of *C. botrys* collected from five different locations in RM are presented in Table 1. Using GC/FID/MS, 75 components were identified representing 90.02 to 91.24% of the total oil content.

Generally, the identified components belonged to four classes of compounds: monoterpene hydrocarbons (MH), oxygen-containing monoterpenes (OM), sesquiterpene hydrocarbons (SH) and oxygen-containing sesquiterpenes (OS). Predominant components were total sesquiterpenes (83.18 to 87.54%) compared to minor quantity of the total monoterpene (2.70 to 6.95%). OS were represented in higher percentage (from 71.38 to 81.81%), followed by a

Table 1. The chemical composition of the essential oil of *Chenopodium botrys* (%)

No	KIL	KIE	Components	Kozuf	Pretor	Strumica	Zletovo	Radovis
1.	924	932.0	α-Thujene	-	-	-	tr	-
2.	939	932.4	α-Pinene	0.20	0.10	0.09	0.08	0.05
3.	946	951.8	Camphene	0.31	0.22		0.44	0.37
4.	969	975.7	Sabinene	3.23	0.08	2.58	2.95	1.81
5.	991	987.9	Myrcene	0.98	4.32	1.40	1.94	1.42
6.	1002	1007.5	α-Phellandrene	-	0.05	-	tr	-
7.	1007	1013.1	Δ^3-carene	-	0.15	0.03	-	0.04
8.	1014	1019.0	α-Terpinene	-	0.10	-	-	-
9.	1022	1026.4	*o*-cymonene	-	0.03	-	-	-
10.	1024	1028.5	Limonene	0.24	0.38	0.17	0.10	0.10
11.	1032	1037.0	β-*Z*-ocimene	-	0.08	-	-	-
12.	1054	1060.2	γ-Terpinene	-	0.03	-	-	-
13.	1065	1067.1	*cis*-Sabinene hydrate	0.05	0.03	-	-	0.04
14.	1083	1089.7	Fenchone	0.20	0.26	0.16	0.19	0.16
15.	1118	1123.4	*cis*-*p*-Menth-2-en-1-ol	0.31	0.32	0.27	0.19	0.30
16.	1136	1142.5	*trans*-*p*-Menth-2-en-1-ol	-	0.05	-	-	-
17.	1154	1158.5	β-Pinene oxide	-	0.11	-	-	-
18.	1165	1170.4	Borneol	-	0.03	-	-	-
19.	1174	1181.0	Terpinene-4-ol	0.06	0.07	0.05	-	0.04
20.	1186	1195.0	α-Terpineol	0.12	0.11	0.06	tr	0.05
21.	1279	1238.5	3*Z*-hexenyl valerate	-	0.02	-	-	-
22.	1284	1290.4	Bornyl acetate	-	0.01	-	-	-
23.	1294	1303.1	*p*-Menth-1-en-9-ol	0.14	0.35	0.12	-	0.14
24.	1335	1342.4	δ-Elemene	0.05	0.05	-	-	-
25.	1346	1342.7	α-Terpenil acetat	-	0.05	tr	-	-
26.	1346	1355.1	α-Cubebene	0.08	0.08	tr	-	-
27.	1374	1382.0	α-Copaene	-	0.09	0.06		
28.	1389	1396.5	β-Elemene	4.21	2.88	1.36	0.99	0.82
29.	1409	1415.9	α-Gurjunene	-	0.07	0.05	tr	tr
30.	1417	1425.9	*trans*-*E*-Caryophyllene	0.17	0.24	0.23	0.12	0.12
31.	1430	1435.6	β-Copaene	-	0.04	-	-	-
32.	1434	1438.4	γ-Elemene	0.38	0.25	0.06	0.05	-
33.	1437	1443.8	α-Guaiene	0.06	0.04	-	-	-
34.	1451	1452.3	*trans*-Muurola-3.5-diene	-	0.12	0.03	-	-
35.	1452	1460.4	α-Hummulene	-	0.09	0.06	-	-
36.	1465	1469.2	*cis*- Muurola-4(14),5-diene	0.09	0.38	0.15	0.11	0.11
37.	1478	1481.1	γ-Muurolene	0.67	0.62	0.31	0.19	-
38.	1485	1493.5	β-Selinene	0.96	0.63	0.28	0.18	0.12
39.	1491	1496.7	10,11-epoxy-Calamenene	0.13	tr	tr	-	-
40.	1498	1501.1	α-Selinene	1.87	1.63	0.78	0.86	0.56
41.	1500	1505.9	α-Muurolene	-	0.49	0.35	0.47	0.25

No	KIL	KIE	Components	Kozuf	Pretor	Strumica	Zletovo	Radovis
42.	1508	1511.1	Germacrene A	0.28	0.19	0.16	0.25	0.31
43.	1513	1519.4	γ-Cadinene	0.23	1.20	0.86	0.83	0.43
44.	1522	1528.3	δ-Cadinene	0.63	1.83	1.08	1.51	0.66
45.	1533	1538.3	*trans*-Cadina-1,4-diene	-	0.19	0.11	0.32	0.16
46.	1537	1543.7	α-Cadinene	0.32	0.43	0.22	0.49	0.21
47.	1545	1547.1	Selina-3,7(11)-diene	0.21	0.19	0.07	-	-
48.	1548	1553.6	Elemol	8.14	9.49	6.31	5.57	5.82
49.	1564	1570.8	β-Calacroene	-	0.07	-	-	-
50.	1567	1576.2	Palustrol	0.99	0.17	0.17	-	0.76
51.	1574	1583.5	α-Cederene epoxide	-	2.64	2.04	2.43	-
52.	1582	1591.4	Cariophyllene oxide	0.42	0.28	0.39	0.44	0.82
53.	1592	1599.4	Viridiflorol	0.23	0.54	0.46	0.77	1.44
54.	1600	1602.5	Guaiol	0.29	-	0.55	0.57	0.92
55.	1609	1611.4	Ledol	0.57	0.84	0.92	0.95	1.45
56.	1622	1637.7	γ-Eudesmol, 10-*epi*	tr	0.46	-	-	tr
57.	1630	1641.8	γ-Eudesmol	2.40	1.71	2.66	2.67	1.57
58.	1642	1647.3	Selina-3,11-dien-6α-ol	8.94	6.42	8.98	8.78	6.57
59.	1649	1656.8	β-Eudesmol	2.52	-	2.96	2.52	2.41
60.	1653	1661.9	α-Cadinol	-	12.11	tr	tr	tr
61.	1658	1662.9	Selin-11-en-4α-ol	11.90	tr	11.96	13.51	9.81
62.	1668	1676.1	Caryophyllene , 14-hydroxy-9-*epi*-(*E*)	1.32	-	1.68	1.55	1.33
63.	1680	1678.7	Elemol acetat	15.41	21.98	12.50	10.79	9.88
64.	1689	1694.1	Botrydiol	2.41	1.21	2.82	1.87	5.73
65.	1700	1702.6	Eudesm-7(11)-en-4-ol	2.26	1.51	2.41	2.84	3.95
66.	1725	1727.0	Guaiol acetat	0.89	0.62	1.15	2.24	9.92
67.	1766	1761.6	β-Costol	1.06	0.71	1.05	1.63	1.89
68.	1777	1775.6	α-Santalol acetate	1.83	-	1.67	1.54	-
69.	1783	1780.8	γ-Eudesmol acetat	0.51	0.41	0.63	0.59	0.92
70.	1794	1791.8	α-Eudesmol acetat	3.51	3.61	3.34	3.88	3.75
71.	1811	1814.9	β-Chenopodiol	1.61	1.20	2.23	2.24	1.85
72.	1855	1859.8	α-Chenopodiol	3.10	2.42	5.43	4.41	3.91
73.	1890	1890.7	β-Chenopodiol-6-acetat	0.91	0.64	1.55	1.66	1.45
74.	1938	1945.4	4α-Acetoxy-eudesman-11- ol	0.42	0.51	0.96	0.83	0.93
75.	1960	1963.9	α-Chenopodiol-6-acetat	2.20	1.90	4.32	4.70	4.73
			Total	90.02	90.13	90.29	91.24	90.08

KIL – Retention index - literature data (Adams, 2007); KIE – Retention index experimentally determined with reference to a homologous series of *n*-alkanes on HP-5ms column (AMDIS), (-) – not found, tr – traces < 0.02;

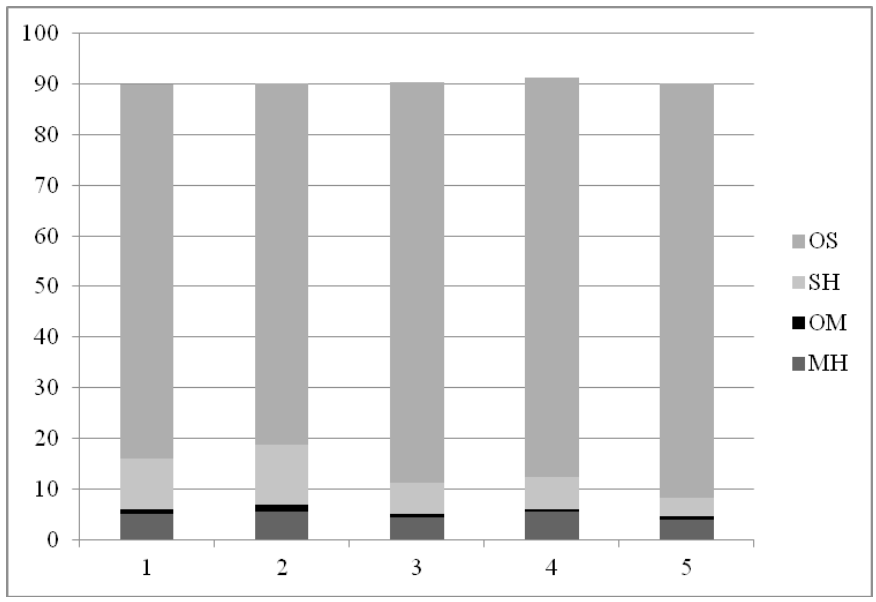

Fig.1. The abundance (%) of different classes of components in the essential oil composition of *Chenopodium botrys* (1- Kozuf, 2 - Pretor, 3 - Strumica, 4 - Zletovo and 5 - Radovis), OS - oxygen containing sesquiterpene; SH - sesquiterpene hydrocarbons; OM - oxygen containing monoterpenes; MH - monoterpene hydrocarbons.

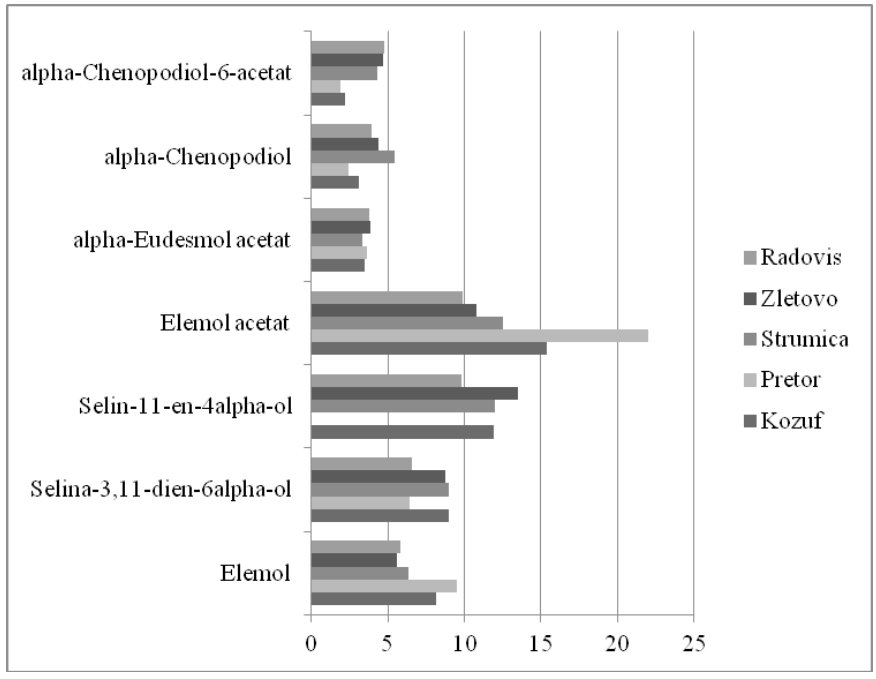

Fig.2. The abundance (%) of main sesquiterpene components in the essential oil of *Chenopodium botrys* (1- elemol; 2 - selina-3,11-dien-6α-ol; 3 - selin-11-en-4α-ol; 4 - elemol acetat; 5 - α-eudesmol acetat; 6 - α-chenopodiol; 7 - α-chenopodiol-6-acetat).

lower content of SH (3.75 to 11.8%). MH were present in a small percentage (1.93 to 5.54%) as well as OM (from 0.38 to 1.41%) (Fig. 1).

Elemol acetate (9.88%-21.98%), saline-11-en-4α-ol (9,81%-13,5%), selina-3,11-diene-6α-ol (6.42%-9.71%) and elemol (5.57%-9.49%) were identified as major OS, followed by a lower content of α-eudesmol acetate (3,24%-4,11%), α-henopodiol (2.42%-5.43%), botrydiol (1.87-5.73%) and α-chenopodiol-6-acetate (1.9%-4.73%) (Table 1, Fig. 2). Traces of α-cadinol were identified in

all essential oil samples except in the sample from Pretor, which contained 12.11% of this component. From the fraction of MH only myrcene (0.73-4.32%) and sabinene (0.08-3.23%) were more present. The essential oil composition was almost constant as the main components were presented in similar percentages in all investigated samples. Difference was noticed only in the sample originating from Pretor, as in this oil elemol acetate was present in higher amount compared to all other oils, while selina-11-en-4α-ol was absent (Fig 2).

The present findings were in correlation with the results obtained in the studies conducted in Greece and North America, showing predominant oxygen-containing sesquiterpenes with elemol acetate (16.3%), elemol (14.1%), botrydiol (11.1%), α-chenopodiol (9.5%), β-eudesmol (7.0%) and selina-3,11-dien-6α-ol (6.1%) as major components for the samples originated from Greece (Tzakou et al., 2007) and α and β-chenopodiol (36%), eudesma-3,11-dien-6α-ol (9.4%), botrydiol (9.0%), elemol (6.5%), elemol acetate (5.5%), γ-eudesmol (5.4%) and α and β-eudesmol (3.7%) as major components in samples originated from North America (Bedrossian et al., 2001). Studies conducted in Iran and Saudi Arabia also showed high content of sesquiterpenes and very low presence of monoterpenes, but with different composition of the predominant components in the oil. In some samples, the most important were 2,3-dehydro-4-oxo-α-ionon (22.4%), (+)-7-*epi*-amiteol (11.5%), elemol (7.4%), α-cadinol (7.0%) and *tau*-cadinol (7.0%) (Mahboubi et al., 2011), but in other predominant were γ-terpineol (52.8%), *p*-cymene (19.0%) and *iso*-ascaridol (7.0%) (Morteza-Semnani and Babanezhad, 2007), or camphor (16.5-25.7%), elemol (14.3-13.4%) and α-cadinol (8.2-11.6%) (Feizbakhsh et al., 2003). Recently, Mokhtari-Karchegani et al. (2014) published different essential oil composition of three ecotypes of *C. botrys* from Iran characterised mainly by monoterpene compounds such as α-pinene, camphene, β-myrcene and 1,8-cineole. One ecotype additionally contained high amounts of β-pinene and camphor.

Evaluation of the biological activity of the essential oil of *C. botrys* was most likely related to the high content of OS with significant antimicrobial activity (Maksimovic et al., 2005; Mahboubi et al., 2011; Tzakou et al., 2007). These findings rationalize further investigations for determination of any antimicrobial activity of essential oil of *C. botrys* from RM. Positive results of this activity could be used in assessment of the antimicrobial potential of this raw material in order to use it in the pharmaceutical and cosmetic industry as well as in food production.

Conclusion

GC/FID/MS analysis of the essential oil composition of *Chenopodium botrys* collected at five different locations in the Republic of Macedonia showed sesquiterpene profile of the oil with major components belonging to the fraction of oxygen-containing sesquiterpenes. The most abundant components were elemol acetate, saline-11-en-4α-ol, selina-3, 11-diene-6α-ol and elemol, followed by lower content α-eudesmol acetate, α-henopodiol, botrydiol and α-chenopodiol-6-acetate.

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Резиме

Хемиски состав на етерично масло од *Chenopodium botrys* L. (Chenopodiaceae)

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Клучни зборови: *Chenopodium botrys*, состав на етерично масло, гасна хроматографија/масена спектрометрија (GC/MS), сесквитерпени

Хемискиот состав на етеричното масло изолирано од надземните делови на *Chenopodium botrys* L. (Chenopodiaceae) собран на 5 различни локалитети во Република Македонија е испитуван со помош на гасна хроматографија во спрега со масена спектрометрија (GC/FID/MS). Идентификувани се 75 компоненти кои претставуваат 90,02-91,24% од целокупното масло. Анализата покажа дека во маслото преовладуваат соединенија од групата на сесквитерпени (83,18-87,54%) со елемол ацетат (9,88%-21,98%), селин-11-ен-4α-ол (9,81%-13,5%), селина-3,11-диен-6α-ол (6,42%-9,71%) и елемол (5,57%-9,49%) како доминантни сесквитерпенски соединенија со кислород, следени со помал процент на α-еудезмол ацетат (3,24%-4,11%), α-хеноподиол (2,42%-5,43%), ботридиол (1,87-5,73%) и α-хеноподиол-6-ацетат (1,9%-4,73%).