SUPPLEMENTARY MATERIAL

Essential Oil Composition of *Ajuga comata* Stapf. from Southern Zagros, Iran

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Abstract

The chemical composition of the essential oil (EO) obtained from the aerial parts of *Ajuga comata* Stapf. was analyzed by GC and GC/MS. Thirty-seven components were identified in the oil. (E)- β -Caryophyllene (30.9 %), caryophyllene oxide (24.9 %), (E)- β -Farnesene (12.6 %), β -Eudesmol (3.2 %), δ -Cadinene (3.1 %) and Germacrene D (3.0 %), were the main compounds in the EOs. The chemical composition of *A. comata* Stapf. from the southern Zagros of Iran is reported for the first time.

Key Words: *Ajuga comata*, Lamiaceae, Essential oil, (E)- β -Caryophyllene.

Experimental

Plant material: Aerial parts of the *Ajuga comata* Stapf. (Syn: *A. chia* Benth.; *A. pseudochia* Schost. and *A. chamaepitys* (L.) Scrb. Subsp. *chia*) were collected in June 2015, from Southern Zagros Mountains of Iran (Bounrood, Near Sepidan City), at the time of flowering. The plant was identified by Fars Research Center for Agriculture. Voucher specimen (No. 11350) was deposited at the Herbarium of Fars Research Center for Agriculture, Shiraz, Iran.

Analysis of the oil: The aerial parts were air-dried at ambient temperature in the shade and hydrodistilled by using a Clevenger-type apparatus for 3 h. The yield of oil was 0.32 % (w/w) and the color of the oil was yellow. It was dissolved in n-hexane (Merck), dried over anhydrous sodium sulfate and stored at 4 °C $\pm$ 2 °C. GC analysis was performed using an Agilent gas chromatograph series 7890-A with a flame ionization detector (FID). The analysis was carried out on fused silica capillary HP-5 column (30 m $\times$ 0.32 mm i.d.; film thickness 0.25 mm). The

injector and detector temperatures were kept at 250 °C and 280 °C, respectively. Nitrogen was used as carrier gas at a flow rate of 1 mL/min; oven temperature program was 60-210 °C at the rate of 4°C/min and then programmed to 240 °C at the rate of 20 °C/min and finally held isothermally for 8.5 min; split ratio was 1:50. GC-MS analysis was carried out by use of Agilent gas chromatograph equipped with fused silica capillary HP-5MS column (30 m × 0.25 mm i.d.; film thickness 0.25 mm) coupled with 5975-C mass spectrometer. Helium was used as carrier gas with ionization voltage of 70 eV. Ion source and interface temperatures were 230 °C and 280 °C, respectively. Mass range was from 45 to 550 amu. Oven temperature program was the same given above for the GC. The constituents of the essential oil were identified by calculation of their retention indices under temperature-programmed conditions for n-alkanes (C8-C25) and the essential oil on a HP-5 column under the same chromatographic conditions. Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those of reported in the literature (Adams 2007). For quantification purpose, relative area percentages obtained by FID were used without the use of correction factors.

Table S1. The relative percentages of essential oil compounds of *Ajuga comata* from the southern Zagros of Iran.

No	Components	Cn	Cn+1	Tx	Sn	Sn+1	RI*	Compounds (%)
1	α -Pinene	9	10	8.297	7.224	10.27	935	tr
2	β -Ocimen	10	11	11.431	10.27	14.06	1031	tr
3	Linalool	10	11	14	10.27	14.06	1098	0.7
4	Terpinene-4-ol	11	12	17.3	14.06	18.21	1178	0.2
5	α -Terpineol	11	12	17.8	14.06	18.21	1190	0.5
6	n-Dodecane	12	13	18.373	18.21	22.41	1204	0.1
7	Pulegone	12	13	19.8	18.21	22.41	1238	1.9
8	Geraniol	12	13	20.505	18.21	22.41	1255	0.3
9	Undecanal	13	14	22.511	22.41	26.48	1303	0.5
10	α -Terpinyl acetate	13	14	24.411	22.41	26.48	1349	0.4
11	Citronellyl acetate	13	14	24.809	22.41	26.48	1359	0.4
12	Eugenol	13	14	24.943	22.41	26.48	1362	0.2
13	Decanoic acid	13	14	25.193	22.41	26.48	1368	0.1
14	α -Copaene	13	14	25.509	22.41	26.48	1376	0.6
15	β -Cubebene	13	14	25.896	22.41	26.48	1386	0.6
16	β -Bourbonene	13	14	26.08	22.41	26.48	1390	0.7
17	(E)- β -Caryophyllene	14	15	27.2	26.48	30.41	1418	30.9
18	β -Copaene	14	15	27.691	26.48	30.41	1431	0.2
19	<i>trans</i> - α -Bergamotene	14	15	27.868	26.48	30.41	1435	0.6
20	(Z)- β -Farnesene	14	15	28.207	26.48	30.41	1444	2.3
21	α -Humulene	14	15	28.595	26.48	30.41	1454	0.5

22	(E)- β -Farnesene	14	15	28.735	26.48	30.41	1457	12.6
23	γ -Gurjunene	14	15	29.3	26.48	30.41	1472	0.4
24	γ -Muurolene	14	15	29.558	26.48	30.41	1478	0.2
25	Germacrene D	14	15	29.773	26.48	30.41	1484	3.0
26	(E)- β -Ionone	14	15	29.93	26.48	30.41	1488	0.4
27	Bicyclogermacrene	14	15	30.32	26.48	30.41	1498	0.7
28	β -Bisabolene	15	16	30.69	30.41	34.16	1508	1.0
29	δ -Cadinene	15	16	31.2	30.41	34.16	1521	3.1
30	<i>cis</i> -Sesquisabinene hydrate	15	16	32.067	30.41	34.16	1544	0.6
31	Dodecanoic acid	15	16	32.907	30.41	34.16	1567	1.4
32	Spathulenol	15	16	33.24	30.41	34.16	1576	2.0
33	Caryophyllene oxide	15	16	33.5	30.41	34.16	1583	24.9
34	Dill apiol	16	17	34.919	34.16	37.73	1621	0.4
35	β -Eudesmol	16	17	36	34.16	37.73	1652	3.2
36	α -Eudesmol	16	17	36.132	34.16	37.73	1655	1.1
37	α -Bisabolol	16	17	37.212	34.16	37.73	1685	1.8
Total								99.5

**RI: Retention indices analyses on HP-5MS column*

Cn: the number of carbon atoms in the smaller n-alkane

Cn+1: the number of carbon atoms in the larger n-alkane

Tx: the retention time of unknown compound

Sn: the retention time of carbon atoms in the smaller n-alkane

Sn+1: the retention time of carbon atoms in the larger n-alkane

tr: traces (less than 0.1 %)

Adams RP. 2007. Identification of essential oil components by gas chromatography/mass spectroscopy. Allured Publishing Corporation, Illinois.