SHORT REPORT

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Chemical Composition of the Essential Oil of *Teucrium flavum* ssp. *flavum* from Zakynthos, Greece

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Abstract: Essential oil extracted from dried aerial parts of *Teucrium flavum* ssp. *flavum* harvested in Zakynthos, Greece, was analysed by gas phase chomatography (GC) and gas chomatography mass spectrometry (GC-MS). The main constituents were caryophyllene (13.5%), caryophyllene oxide (8.5%), 4-vinyl guaiacol (6.0%) and α-humulene (5.0%).

Keywords: *Teucrium flavum*; essential oil; GC-MS; caryophyllene; caryophyllene oxide; 4-vinyl guaiacol; α-humulene.

1. Plant Source

Teucrium flavum L. (Lamiaceae) is an evergreen perennial subshrub up to 60 cm known in Greece as "Chamaidrya", "Moskhokhortaro" and "Dontokhorti". It is characterized by pubescent stems and yellow corolla assembled in terminal spikes.

An infusion of the aerial parts has been used in folk medicine orally as an antidiabetic and externally as an astringent to heal skin eruptions and wounds [1]. Different extracts showed a significant anti-inflammatory effect [2].

In continuation of our studies on Lamiaceae of the Mediterranean area [3-6] the aerial parts of *T. flavum* were collected in July, 2008, in Zakynthos, Greece. The identification was done by Prof. F.M. Raimondo, University of Palermo, Italy.

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2. Previous Studies

The presence of diterpenoids has been previously reported [7]. The essential oils from plants growing in Croatia, Corsica, Serbia and Montenegro, Iran and Greece (East Thessaly and Milopotamos) were also studied [1, 8-11].

3. Present Study

The oil from air-dried and ground aerial parts of *T. flavum* was isolated by hydrodistillation for 3 h, using a Clevenger-type apparatus according to the method recommended in the European Pharmacopoeia [12]. The oil was dried over anhydrous sodium sulphate and stored under N_2 at +4°C in the dark until tested and analysed. The sample yielded 0.13% of yellow oil (w/w), with a pleasant smell.

GC analysis: Analytical gas chromatography was carried out on a Perkin-Elmer Sigma 115 gas chromatograph fitted with a HP-5 MS capillary column (30 m x 0.25 mm i.d.; 0.25 μm film thickness). Helium was the carrier gas (1 mL min^{-1}). Column temperature was initially kept at 40°C for 5 min, then gradually increased to 250°C at 2°C min^{-1}, held for 15 min and finally raised to 270°C at 10°C min^{-1}. Diluted samples (1/100 v/v, in *n*-hexane) of 1 μL were injected manually at 250°C, and in the splitless mode. Flame ionization detection (FID) was performed at 280°C. Analysis was also run by using a fused silica HP Innowax polyethylenglycol capillary column (50 m x 0.20 mm i.d.; 0.20 μm film thickness).

GC-MS analysis: GC-MS analysis was performed on an Agilent 6850 Ser. II apparatus, fitted with a fused silica HP-1 capillary column (30 m x 0.25 mm i.d.; 0.33 μm film thickness), coupled to an Agilent Mass Selective Detector MSD 5973; ionization voltage 70 eV; electron multiplier energy 2000 V. Gas chromatographic conditions were as reported above; transfer line temperature, 295°C. Analysis was also run by using a fused silica HP Innowax polyethylenglycol capillary column (60 m x 0.25 mm i.d.; 0.33 μm film thickness) [13].

Qualitative and quantitative analyses: Most constituents were identified by gas chromatography by comparison of their retention indices (Ri) with either those of the literature [14, 15] or with those of authentic compounds available in our laboratories. The retention indices were determined in relation to a homologous series of *n*-alkanes (C_8-C_{24}) under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with either those stored in NIST 02 and Wiley 275 libraries or with mass spectra from the literature [14, 16] and our home made library. Component relative concentrations were calculated based on GC peak areas without using correction factors.

Sixty-two compounds were determined in the reported essential oil, representing 89.7% of total oil content. The retention indices, percentage composition and identification methods are given in Table 1, where the components are listed in order of elution from a HP-5 MS column. The main constituents were found to be caryophyllene (13.5%), caryophyllene oxide (8.5%), 4-vinyl guaiacol (6.0%) and α-humulene (5.0%). Sesquiterpenes constituted the most abundant fraction of the oil (49.1%), with a prevalence of sesquiterpene hydrocarbons (41.3%) among which caryophyllene (13.5%) and α-humulene (5.0%) predominated. Among the 10 oxygen containing sesquiterpenes (14.4%), caryophyllene oxide (8.5%) was the most abundant.

As regards monoterpenes, 9 oxygen containing monoterpenes accounted for the 7.5% of the total oil, with linalool (2.9%) as main compound, while terpinolene (0.5%) was the only monoterpene hydrocarbon. Also phenolic fraction was noteworthy (10.3%), with 4-vinyl guaiacol (6.0%) being the main compound.

Caryophyllene, the main compound in our oil, was abundant also in the oils of *T. flavum* from Greece (East Thessaly) (22.2%) [1], Iran (30.6%) [4] and Croatia (15.8%) [10], while it was present in minor concentrations in *T. flavum* from Serbia and Montenegro (5.4%) [9]. As regards the other compounds, there is a great variability in the compositions of the oils, depending on the subspecies

and the geographical position. Our previous papers on the analysis of the essential oils of *Teucrium* spp. showed that the sesquiterpenes group is usually dominant, although the main component may vary [11, 17-19]. Besides, the concentrations of caryophyllene and caryophyllene oxide are usually very high in the essential oils of numerous *Teucrium* species [19]. The present results concur with these findings.

Table 1. Essential oil composition of *T. flavum* (%)

R_i [a]	R_i [b]	Compound	Identification [c]	% [d]
977	1452	1-Octen-3-ol	R_i, MS	T
1086	1265	Terpinolene	R_i, MS	0.5
1098	1553	Linalool	R_i, MS	2.9
1099	1387	Isoamyl isovalerate	R_i, MS	0.1
1132	1653	*cis*-Pinocarveol	R_i, MS	0.6
1138	1664	*trans*-Pinocarveol	R_i, MS	0.7
1144	1663	*cis*-Verbenol	R_i, MS	0.8
1148	1737	Veratrole	R_i, MS	1.5
1176	1611	Terpineol-4	R_i, MS, Co-GC	0.9
1196	1804	Myrtenol	R_i, MS	0.6
1217	1845	*trans*-Carveol	R_i, MS	0.1
1232	2012	*p*-Anisaldehyde	R_i, MS, Co-GC	T
1241	1752	Carvone	R_i, MS	0.8
1285	2067	*p*-Cymen-7-ol	R_i, MS	0.1
1290	2198	Thymol	R_i, MS, Co-GC	0.7
1297	2239	Carvacrol	R_i, MS, Co-GC	1.3
1313	**2180**	**4-Vinyl guaiacol**	**R_i, MS**	**6.0**
1352	1466	α-Cubebene	R_i, MS	T
1353	2186	Eugenol	R_i, MS, Co-GC	0.8
1377	1497	α-Copaene	R_i, MS	3.3
1380	1836	(*E*)-β-Damascenone	R_i, MS	0.9
1385	1535	β-Bourbonene	R_i, MS	2.6
1418	**1612**	**Caryophyllene**	**R_i, MS**	**13.5**
1435	1573	(*Z*)-α-*trans*-Bergamotene	R_i, MS	1.7
1437	1675	β-Humulene	R_i, MS	0.3
1452	1673	β-Farnesene		1.3
1455	1689	α-Humulene	R_i, MS	5.0
1463	1661	*allo*-Aromadendrene	R_i, MS	1.0
1477	1726	Germacrene D	R_i, MS	1.5
1478	1704	γ-Muurolene	R_i, MS	1.5
1482	1957	(*E*)-β-Ionone	R_i, MS, Co-GC	0.9
1498	1638	*epi*-Bicyclosesquiphellandrene	R_i, MS	0.6
1503	1740	α-Muurolene	R_i, MS	0.3
1506	1760	(*E*,*E*)-α-Farnesene	R_i, MS	0.4
1510	1743	β-Bisabolene	R_i, MS	1.1
1513	1709	Ledene	R_i, MS	0.3
1515	1776	γ-Cadinene	R_i, MS	1.7
1526	1773	δ-Cadinene	R_i, MS	2.9
1541	1942	α-Calacorene	R_i, MS	1.2
1551	1942	β-Calacorene	R_i, MS	1.1
1578	2150	Spathulenol	R_i, MS	0.5
1580	**2008**	**Caryophyllene oxide**	**R_i, MS**	**8.5**
1588	2098	Globulol	R_i, MS	0.6
1591	2104	Viridiflorol	R_i, MS	1.5

1598	2108	Guaiol	R_i, MS	0.4
1605	2071	Humulene epoxide II	R_i, MS	1.7
1608	2098	β-Oplopenone	R_i, MS	T
1640	2316	Caryophylladienol I	R_i, MS	T
1643	2209	T-Muurolol		T
1652	2255	α-Cadinol	R_i, MS	1.2
1723	1989	Tetradecanoic acid methyl ester	R_i, MS, Co-GC	0.1
1762	2655	Benzyl benzoate	R_i, MS, Co-GC	0.5
1845	2131	Hexahydrofarnesyl acetone	R_i, MS	3.4
1929	2208	Hexadecanoic acid methyl ester	R_i, MS, Co-GC	1.9
1950	2622	(*Z*)-Phytol	R_i, MS	1.9
1957	2931	Hexadecanoic acid	R_i, MS, Co-GC	1.2
2094	2436	(*Z*)-9-Octadecenoic acid methyl ester	R_i, MS, Co-GC	0.6
2300	2300	Tricosane	R_i, MS, Co-GC	0.7
2400	2400	Tetracosane	R_i, MS, Co-GC	0.4
2500	2500	Pentacosane	R_i, MS	2.2
2700	2700	Heptacosane	R_i, MS	0.9
2829		Squalene		T

TOTAL	**89.7**
Monoterpene hydrocarbons	**0.5**
Oxygenated monoterpenes	**7.5**
Sesquiterpene hydrocarbons	**41.3**
Oxygenated sesquiterpenes	**14.4**
Fatty acids and esters	**7.8**
Phenols	**10.3**
Hydrocarbons	**4.2**
Carbonylic compounds	**1.8**
Others	**1.9**

R_i^a: Retention index on a HP-5MS column; R_i^b: Retention index on a Innowax column; [c]: Ri = retention index identical to bibliography; MS = identification based on comparison of mass spectra; Co-GC = retention time identical to authentic compounds; [d]: t = trace, less than 0.05 %.

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