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RESEARCH ARTICLE

Microdistilled essential oil of *Thymus integer* Griseb., endemic in Cyprus

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Abstract
The aim of the present study was to characterize the essential oil composition of *Thymus integer* Griseb. (Lamiaceae), an endemic species in Cyprus. Microdistilled essential oil was analysed simultaneously both by Gas Chromatography-Flame Ionization Detector (GC-FID), and Gas Chromatography/Mass Spectrometry (GC/MS). Overall, 49 components were determined and the major components were characterized as borneol (22%), *p*-cymene (10.1%), γ–terpinene (9.6%), α-pinene (8.7%), camphene (7.7%), and terpinen-4-ol (5.4%), respectively.

Keywords: *Thymus integer*, microdistillation, essential oil, GC-FID, GC-MS

Introduction

The genus *Thymus* L. is represented in Cyprus by two species, *T. capitatus* L. and *T. integer* Griseb. (Meikle, 1985). Traditionally, both are used to treat respiratory and digestive disorders (González-Tejero et al, 2008). *T. integer* is locally known as "*thymari*" and endemic in Cyprus (Bellomaria et al., 1994). It grows at 80-1700 m altitude, and flowering stage is from February to June. *T. integer* infusion prepared from aerial parts is internally used as spasmolytic, carminative, stomachic, and against cough and cold. Externally, the extracts are used as incense against headaches. Moreover, due to antiseptic and astringent properties, compresses prepared from this plant are used for the treatment of burns and external wounds (Arnold, 1985). The essential oils of the genus *Thymus* have been well reviewed (Stahl-Biskup, 1991; Başer, 1992; Bellomaria et al., 1994; Başer & Kırımer, 2006; Özkum Yavuz et al., 2017; Başer & Kırımer, 2018).

The aim of this study was to characterize the essential oil composition of *Thymus integer* Griseb. endemic in Cyprus obtained by microdistillation.

Materials and Methods

Plant material

Aerial parts of the plant material, growing wild in Cyprus, was collected during the flowering stage at Madari in Southern Cyprus. A voucher specimen is kept at the Herbarium of the Near East University, Turkish Republic of Northern Cyprus (NEUN6905).

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Microdistillation

The volatiles were obtained after microdistillation of the dried plant material (1 g) using an Eppendorf MicroDistiller® containing 10 mL of distilled water per sample vial. The sample vial was heated to 108°C at a rate of 20°C/min for 90 min followed by heating at 112°C at the rate of 20°C/min for 30 min. The sample was subjected to a final post-run for 2 min under the same conditions. The collecting vial, containing a solution of NaCl (2.5 g) and water (0.5 ml) plus *n*-hexane (350 µl) to trap volatile components, which were cooled to –5°C during distillation. Thereafter, the organic layer in the collection vial was separated and analysed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) simultaneously.

GC-MS analysis

The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 0.25 µm film thickness) was used with helium as carrier gas (0.8 ml/min). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. Split ratio was adjusted at 40:1. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 35 to 450.

GC analysis

The GC analysis was carried out using an Agilent 6890N GC system. FID detector temperature was 300°C. To obtain the same elution order with GC-MS, simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms. The analysis results are given in Table I.

Identification of the essential oil components were carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library, MassFinder Software 4.0) (McLafferty & Stauffer, 1989; Koenig, Joulain & Hochmuth, 2004) and in-house "Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils.

Results and Discussion

The essential oil of *T. integer* obtained by microdistillation and analysed by GC-FID and GC-MS yielded 49 compounds representing 99.3% of essential oil. The major components were identified as borneol (22%), *p*-cymene (10.1%), γ-terpinene (9.6%), α-pinene (8.7%), camphene (7.7%) and terpinen-4-ol (5.4%). Thymol content was determined as 2.9% while the carvacrol content was 0.3% (Table 1.).

Table 1. The Essential Oil Composition of *Thymus integer*

RRI	Compound	%	IM
1014	Tricyclene	0.4	t_R, MS
1032	α-Pinene	8.7	t_R, MS
1035	α-Thujene	0.8	t_R, MS
1076	Camphene	7.7	t_R, MS
1118	β-Pinene	2.9	t_R, MS
1132	Sabinene	1.5	t_R, MS
1174	Myrcene	0.7	t_R, MS
1176	α-Phellandrene	0.5	t_R, MS
1188	α-Terpinene	2.0	t_R, MS

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1203	Limonene	2.0	t_R, MS
1218	β-Phellandrene	1.4	t_R, MS
1255	γ-Terpinene	9.6	t_R, MS
1280	*p*-Cymene	10.1	t_R, MS
1290	Terpinolene	0.8	t_R, MS
1452	1-Octen-3-ol	0.5	MS
1474	*trans*-Sabinene hydrate	0.2	MS
1479	(*E,Z*)-2,4-Heptadienal	tr	MS
1499	α-Campholene aldehyde	tr	MS
1507	(*E,E*)-2,4-Heptadienal	0.1	MS
1532	Camphor	1.1	t_R, MS
1553	Linalool	1.6	t_R, MS
1556	*cis*-Sabinene hydrate	0.2	MS
1562	Octanol	0.1	t_R, MS
1571	*trans-p*-Menth-2-en-1-ol	0.2	MS
1591	Bornyl acetate	2.7	t_R, MS
1611	Terpinen-4-ol	5.4	t_R, MS
1612	β-Caryophyllene	2.5	t_R, MS
1638	*cis-p*-Menth-2-en-1-ol	0.1	MS
1683	*trans*-Verbenol	0.2	t_R, MS
1687	α-Humulene	0.4	t_R, MS
1706	α-Terpineol	1.4	t_R, MS
1719	Borneol	22.0	t_R, MS
1726	Germacrene D	2.1	MS
1751	Carvone	0.2	t_R, MS
1755	Bicyclogermacrene	0.7	MS
1758	(*E,E*)-α-Farnesene	0.2	MS
1773	δ-Cadinene	0.6	MS
1845	*trans*-Carveol	tr	t_R, MS
1857	Geraniol	0.2	t_R, MS
1864	*p*-Cymen-8-ol	0.3	t_R, MS
2008	Caryophyllene oxide	1.5	t_R, MS
2144	Spathulenol	0.7	MS
2187	*T*-Cadinol	0.2	MS
2198	Thymol	2.9	t_R, MS
2239	Carvacrol	0.3	t_R, MS
2255	α-Cadinol	0.5	MS
2316	Caryophylla-2(12),6(13)-dien-5β-ol (=*Caryophylladienol I*)	0.2	MS
2324	Caryophylla-2(12),6(13)-dien-5α-ol (=*Caryophylladienol II*)	0.6	MS
2392	Caryophylla-2(12),6-dien-5β-ol (=*Caryophyllenol II*)	0.3	MS
	Total	99.3	

RRI: Relative retention indices calculated against *n*-alkanes, % calculated from FID data, tr: Trace (< 0.1 %), IM: Identification method, tR, identification based on the retention times of genuine compounds on the HP Innowax column; MS, identified on the basis of computer matching of the mass spectra with those of the Wiley and MassFinder libraries and comparison with literature data.

Bellomaria et al. (1994) reported the major compounds of *T. integer* essential oil as *p*-cymene (15.7-25%), borneol (18.7-23.8%), γ-terpinene (9.2-12.3%), thymol (3.1-8.4%) and carvacrol (0.5-1.6%), respectively. The authors had collected plant materials from six locations in Southern Cyprus studying leaf, flower and fruit oils. They reported the oil yields in the range of 0.2-0.9%. Our material was collected from a different location. We had also found borneol, *p*-cymene and γ-terpinene as main components. The contents of thymol and carvacrol which are typical thyme/ oregano oil constituents were low in our sample as well. This study confirms the previous study in characterizing the oil composition of *Thymus integer,* which is endemic in Cyprus.

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