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Original article

COMPOSITION OF THE ESSENTIAL OIL OF *CALAMINTHA GRANDIFLORA* (L.) MOENCH

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Abstract

In this study, the aerial parts of Calamintha grandiflora (L.) Moench collected from two different location of Turkey were distilled using Eppendorf Microdistiller® and analyzed by GC and GC/MS. Isopinocamphone (35.4 %), pinocamphone (8.4 %) and linalool (7.6 %) were detected as main constituents in sample A; β-pinene (19.4%), isopinocamphone (19.0%), pinocamphone (15.1%) and limonene (12.1%) were found as main constituents in sample B.

Key words: *Calamintha grandiflora, Labiatae, Essential oil composition*

Calamintha grandiflora (L.) Moench'nın Uçucu Yağ Bileşimi

Bu çalışmada Calamintha grandiflora (L.) Moench'nın toprak üstü kısımlarının mikrodistilasyon (Eppendorf Microdistiller) ile uçucu yağları elde edilmiş ve GC ve GC/MS analizleri yapılmıştır. Numune A'da izopinokamfon (% 35.4), pinokamfon (% 8.4) ve linalool (% 7.6) ana bileşik olarak bulunmuştur. Numune B'de β-pinen (% 19.4), izopinokamfon (% 19.0), pinockamfon (% 15.1) ve limonen (% 12.1) ana bileşik olarak bulunmuştur

Anahtar kelimeler: *Calamintha grandiflora, Labiatae, Uçucu yağ bileşikleri*

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INTRODUCTION

The genus *Calamintha* Miller (Lamiaceae) is distributed in Europe, Eastern Mediterranean region, Central Asia, North Africa and Americas (1, 2). In Turkey it is represented by 9 species and 12 taxa, five being endemic to Turkey. The ratio of endemism is over 45% (3-5). *Calamintha pamphylica* Boiss. & Heldr. subsp. *alanyense* S. Alan & A. Ocak has been publicated in 2007 (6).

Calamintha is known as in Turkish "Güzel Nane, Dağ Nanesi, Miskotu, Tıbbi Miskotu, Yabani Oğulotu'' and used as folk medicine in Turkey. It also has horticultural uses (1,7). *Calamintha* species are used as stimulant, antispasmodic, emmenagogue, digestive, antiseptic, diaphoretic, expectorant and for strengthening central nervous system (1, 2, 8). They are also used for stomach and throat ache, kidney disorders and as spice (9, 10).

The oil of *C. grandiflora* was previously investigated. In the oil of Greek origin (1.8% yield) pulegone (35.2%), menthone (20.2%) and isomenthone (15.2) were found as main constituents (11). The oil of French origin contained as main constituents pulegone (27.6%), isomenthone (24.7%), neo-isomenthone (23.7%) and menthol (10.8%) (12). In the oil of Turkish origin collected from Kütahya: Domaniç-Daritepe region, isopinocamphone (52.6%) was found as the main constituent (13).

The purpose of the present study was to confirm the essential oil composition of *Calamintha grandiflora* of Turkish origin and find out if pulegon chemotype exists in Turkey.

EXPERIMENTAL

Plant material

In the present study, aerial parts of *Calamintha grandiflora* (L.) Moench were collected from the following places shown as below.

A: Eskişehir; Efsunbaba, 14.08.2002 ESSE:14413

B: Kütahya; Domaniç, 18.07.2001 ESSE:14380

Essential oil distillation

Essential oils were distilled from 500 mg of aerial parts of *Calamintha grandiflora* using an Eppendorf Microdistiller®. The fruits were placed in a sample vial together with 10 mL of water. Sodium chloride (2 g) and water (0.5 mL) were placed in the collecting vial. *n*-Hexane (300 µL) was added to the collecting vial to trap volatile compounds. The apparatus was operated according to Essential Oils Programme. Sample vials were heated to 100 °C at a rate of 20 °C/min, kept at 100 °C for 15 min, then heated to 112 °C at a rate of 20 °C/min, and kept at 112 °C for 35 min. Finally, the sample was subjected to a post-run for 2 min under the same conditions. Collecting vials placed in cooler, were kept at −1°C during distillation. After complete the distillation, the organic layer in the collection vial was separated from water phase and injected into the GC and GC/MS.

GC and GC/MS conditions

The oils were analyzed by capillary GC and GC/MS using a Agilent GC-MSD system (Agilent Technologies Inc., Santa Clara,CA).

GC/MS: The GC/MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60m x 0.25mm, 0.25µm 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, at splitless mode. The injector temperature was at 250°C. MS were taken at 70 eV. Mass range was from m/z 35 to 450.

GC: The GC analysis was carried out using an Agilent 6890N GC system. In order to obtain same elution order with GC/MS, simultaneous injection was done by using same column and an appropriate operational conditions. FID temperature was 300°C.

The components of essential oils were identified by comparison of their mass spectra with those in the Baser Library of Essential Oil Constituents, Wiley GC/MS Library, Adams Library, MassFinder Library and confirmed by comparison of their retention indices. Alkanes were used as reference points in the calculation of relative retention indices (RRI). Relative percentage amounts of the separated compounds were calculated from FID chromatograms. The results of analysis are shown in Table 1.

RESULTS AND DISCUSSION

The oils were distilled from aerial parts of *Calamintha grandiflora* using Eppendorf Microdistiller® and analyzed by GC and GC/MS.

Isopinocamphone (35.4 %), pinocamphone (8.4 %) and linalool (7.6 %) were detected as main constituents in sample A; β-pinene (19.4%), isopinocamphone (19.0%), pinocamphone (15.1%) and limonene (12.1%) were found as main constituents in sample B.

Our results compare well with the previous study on the Turkish oil in which isopinocamphone was found as the main constituent. Isopinocamphone is rarely found in nature. It is the main constituent in the oils of *Hyssopus officinalis* L. (22-62%) (14-17), *Cyclotrichium stamineum* (Boiss. & Hohen.) Manden. & Scheng. (13.7%) (18), *Sphaeranthus suaveolens* DC. (33.5%) (19), *Artemisia alba* Turra (24.6%) (20), *Cistus ladanifer* L. (10.5%) (21), chemotypes of *Mentha aquatica* L. (22, 23) and *Mentha citrata* x *M. crispa* and *M. citrata* x *M. aquatica* hybrids (24). Pinocamphone has been reported as a main constituent in the oils of *Artemisia annua* L. (15%) (25), *Cyclotrichium stamineum* (34%) (18), *Dracocephalum nutans* L. (40%) (26), *Hyssopus officinalis* L. (44 and 69%) (27, 28).

Table 1. The Composition of the Essential Oils of *Calamintha grandiflora*

		Calamintha grandiflora	
RRI	**Compounds**	**A %**	**B %**
1032	α-Pinene	tr	tr
1118	**β-Pinene**	5.6	**19.4**
1174	Myrcene	tr	tr
1188	α-Terpinene	tr	tr
1203	**Limonene**	5.2	**12.1**
1255	γ-terpinene	1.7	2.5
1280	*p*-Cymene	0.7	1.0
1290	Terpinolene	1.1	0.5
1450	trans-Linalool oxide (*Furanoid*)	1.1	--
1478	*cis*-Linalool oxide (*Furanoid*)	0.2	--
1482	(*Z*)-3-Hexenyl-2-methyl butyrate	0.3	--
1505	Dihydroedulane II	0.4	--
1536	**Pinocamphone**	**8.4**	**15.1**

1553	**Linalool**	**7.6**	2.9
1562	**Isopinocamphone**	**35.4**	**19.0**
1586	Pinocarvone	tr	0.6
1597	Nopinone	tr	0.9
1611	Terpinen-4 ol	2.9	3.7
1612	β-Caryophyllene	0.3	0.6
1648	Myrtenal	3.4	2.6
1662	Pulegone	0.3	0.6
1664	*trans*-Pinocarveol	0.3	1.2
1700	*p*-Mentha-1,8-dien-4-ol (=*Limonen-4-ol*)	0.4	tr
1706	α-Terpineol	4.0	2.6
1751	Carvone	2.6	tr
1804	Myrtenol	6.9	3.4
1838	(*E*)-β-Damascenone	0.4	1.2
1857	Geraniol	0.7	tr
1845	trans-Carveol	tr	1.0
2008	Caryophyllene oxide	0.5	1.0
2130	Spathulenol	0.8	tr
2131	Hexahydrofarnesyl acetone	0.6	tr
2380	Dihydroactinidiolide	--	0.8
2384	Hexadecanol	tr	2.3
	Total	**92.1**	**91.3**

RRI Relative retention indices calculated against n-alkanes
% calculated from FID data
tr Trace (< 0.1 %)

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