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MICRODISTILLATION AS A USEFUL TOOL FOR THE ANALYSIS OF MINUTE AMOUNTS OF AROMATIC PLANT MATERIALS*

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The MicroDistiller (Eppendorf), enables the distillation of aromatic material even in very small quantities. Using the simple microdistillation technique, minute amounts of Cyclotrichium leucotrichum *(Stapf ex Rech. fil.) Leblebici and* C. stamineum *(Boiss. and Hohen.) Manden. and Scheng. were investigated for their volatiles by GC/MS. The main constituents were identified as* β*-caryophyllene (14%), camphor (12%), and* p*-menth-3-en-8-ol (11%) for* C. leucotrichum*, and pinocamphone (33%), isopinocamphone (13%), and myrtenyl acetate (9%) for* C. stamineum.

Key words: *Cyclotrichium*, Lamiaceae, microdistillation, volatile compounds, GC/MS analysis.

The Lamiaceae is an important family with numerous essential oil-rich aromatic plants. The small Asiatic genus *Cyclotrichium* is represented by five species of which two are endemic to Turkey. *Cyclotrichium leucotrichum* (Stapf ex Rech. fil.) Leblebici and *C. stamineum* (Boiss. and Hohen.) Manden. and Scheng. grow mainly in eastern Anatolia as reported in the Flora of Turkey [1].

C. niveum (Boiss.) Manden. and Scheng. and *C. origanifolium* (Labill.) Manden. and Scheng., two other Turkish species, are used to make herbal teas. Their essential oils were studied by us and pulegone was found to be the main constituent [2-4].

Here, we report on the GC/MS analysis of steam volatiles obtained by microdistillation from samples taken from herbarium specimens.

As previously reported, microdistillation is used for obtaining volatiles for subsequent analysis of plant materials in minute amounts [5-7]. The microdistillation technique used in this study enabled six simultaneous distillations on a bench-top without the use of water for cooling.

The GC/MS analysis of the steam volatiles resulted in the identification of thirty-two compounds in *C. leucotrichum* representing 89.5% of the total with *β*-caryophyllene (14.1%), camphor (11.9%), and *p*-menth-3-en-8-ol (11.4%) as the main constituents. Twelve compounds were characterized in the steam volatiles of *C. stamineum* representing 92.7% of the total with pinocamphone (33.8%), isopinocamphone (13.7%), and myrtenyl acetate (9.1%) as the main constituents.

The volatiles of *C. leucotrichum* and *C. stamineum* showed a totally different composition as compared to those of *C. niveum* and *C. origanifolium* which were characterized by large amounts in their essential oils [3, 4].

The results demonstrate convincingly that microdistillation and the subsequent GC/MS analysis can safely be used to investigate the composition of volatiles in minute samples of aromatic plants even using herbarium specimens.

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TABLE 1. Composition of the Essential Oils of *Cyclotrichium* Species

RRI	Compound	A, %	B, %	RRI	Compound	A, %	B, %
1032	α-Pinene	-	0.99	1612	β-Caryophyllene	**14.14**	-
1058	3-Hexanone	0.34	-	1621	*p*-Menth-3-en-8-ol	11.44	-
1118	β-Pinene	0.55	5.52	1623	Octyl butyrate	0.63	-
1202	3-Hexanol	0.29	-	1662	Pulegone	0.31	-
1203	Limonene	4.13	-	1687	α-Humulene	0.66	-
1213	1,8-Cineole	9.26	-	1704	Myrtenyl acetate	-	9.11
1255	γ-Terpinene	0.26	2.68	1719	Borneol	1.53	1.75
1270	*p*-Mentha-3,8-diene	1.45	-	1755	Bicyclogermacrene	0.90	-
1280	*p*-Cymene	3.03	5.25	1763	Naphthalene	3.86	7.36
1348	6-Methyl-5-hepten-2-one	0.49	-	1773	β-Cadinene	0.84	-
1406	α-Fenchone	5.95	-	1804	Myrtenol	-	3.35
1532	Camphor	11.92	-	1838	(*E*)-β-Damascenone	1.35	-
1536	Pinocamphone	-	**33.78**	1864	*p*-Cymen-8-ol	0.79	-
1541	Neomenthyl acetate	2.21	-	1868	(*E*)-Geranyl acetone	1.37	-
1562	Isopinocamphone	-	13.67	2008	Caryophyllene oxide	1.41	-
1574	Menthyl acetate	1.08	-	2088	1-*epi*-Cubenol	0.29	-
1597	Bornyl acetate	0.54	-	2187	T-Cadinol	3.81	-
1604	Neomenthol	1.63	-	2198	Thymol	0.76	-
1611	Terpinen-4-ol	-	7.22	2239	Carvacrol	2.28	1.99
				Total		89.5	92.67

A: Cyclotrichium leucotrichum; B: Cyclotrichium stamineum; RRI: Relative retention indexes calculated against n-alkanes; %: calculated from TIC data.

EXPERIMENTAL

Plant Material. A few dried leaves of *C.leucotrichum* (K. 446) and *C. stamineum* (D23924) were obtained from the herbarium of the Royal Botanic Garden, Edinburgh (E).

Distillation Method. The volatiles were obtained by microdistillation of the *C. leucotrichum* (67 mg) and *C. stamineum* (82 mg) materials using an Eppendorf MicroDistiller®.

Crushed plant material was added to a sample vial containing 10 mL distilled water. NaCl (2.5 g) and water (0.5 mL) was placed in the collecting vial. *n*-Hexane (350 μL) was added to the collecting vial to trap the volatile components. The sample vial was heated to 108°C at a rate of 20°C/min and kept at 108°C for 90 min, then, heated to 112°C at a rate of 20°C/min and kept at this temperature for 30 min. Finally the sample was subjected to post-run for 2 min under the same conditions. The collecting vial was cooled to –5°C during distillation. After the distillation was completed, the organic layer in the collection vial was separated and injected into the GC/MS system.

Analysis of the Volatiles. The volatiles were analyzed using a Hewlett-Packard G1800A GCD system. HP-Innowax FSC column (60 m × 0.25 mm ⌀, with 0.25 μm film thickness). Helium (0.8 ml/min) was used as carrier gas.

GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min and then kept constant at 220°C for 10 min to 240°C at rate of 1°C/min. Mass range was recorded from *m/z* 35 to 425. Injections were applied splitless. Injection port temperature was at 250°C. MS were recorded at 70 eV. Relative percentage amounts of the separated compounds were calculated automatically from peak areas of the total ion chromatogram (TIC). Alkanes were used as reference points in the calculation of relative retention indexes (RRI). Library search was carried out using both “Wiley GC/MS Library” and “TBAM Library of Essential Oil Constituents”.

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