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ANALYSIS OF VOLATILE COMPONENTS FROM *Tanacetum cadmeum* BY HYDRODISTILLATION AND DIRECT THERMAL DESORPTION METHODS USING GC×GC-TOF/MS

Fahrettin Gogus,[1] Ali Celik,[2] Alastair C. Lewis,[3] and Mustafa Z. Ozel[4*]

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The genus *Tanacetum* with ca. 200 species is widespread mainly in Europe, Asia, and North America [1–3]. The plant is 30–150 cm tall [3, 4] and has a hairy stem and woody roots and branches. It is an aromatic perennial plant. *Tanacetum* extracts have been widely used against intestinal worms, kidney disease, and respiratory infections [2]. They are also natural antioxidants [5]. *Tanacetum* species have been used since ancient times. They have traditionally been used as a repellent and deterrent against insects and to alleviate the symptoms of migraine, arthritis, and psoriasis [6]. The dynamic headspace technique is a very popular method for analyzing volatile compounds in food [7] and plant materials [8]. Direct thermal desorption (DTD) is one of the dynamic headspace techniques with cryogenic trapping. This technique allows for the qualitative and/or quantitative analysis of volatile compounds with little or no sample preparation [9]. The main objectives of this study are to characterize the composition of the volatile fractions obtained from the leaves and flowers of Turkish endemic *T. cadmeum* analyzed by GC×GC-TOF/MS and to carry out a comparative evaluation of its composition with respect to the isolation techniques, namely HD and DTD. Reviewing the literature, it appears that the chemical composition of the essential oil of *T. cadmeum* has not been investigated before.

Tanacetum cadmeum (Boiss.) Heywood ssp. *cadmeum* was collected at the flowering stage (June 2007) from the Honaz Mountain, Denizli, Turkey. Air-dried leaves and flowers were subjected to hydrodistillation in a Clavenger apparatus for 3 h. The GC×GC-TOF/MS system was equipped with a dual stage commercial thermal desorption injector, incorporating a thermal desorption unit (TDU), connected to a programmable-temperature vaporization (PTV) injector, CIS-4 plus (Gerstel), by a heated transfer line. The injector was equipped with an MPS autosampler (Gerstel) able to handle the program for 98 thermal desorption tubes. Initial desorption is carried out at 150°C using the TDU for 3 min under a helium flow of 1.5 mL/min in the splitless mode while maintaining a cryofocussing temperature of 20°C in the PTV injector of the GC–MS system. After cooling of the TDU to 40°C, the programmable temperature vaporization system is ramped to a final temperature of 200°C and the analytes are transferred to the GC column. The first column was a nonpolar DB5 (5% phenyl–95% methyl polysiloxane, 28.8 m × 0.32 mm i.d. × 0.25 μm film thickness) and the second column, a DB17 (50% methyl–50% phenyl polysiloxane, 2.1 m × 0.10 mm i.d. × 0.10 μm film thickness). Both columns were purchased from J & W Scientific (Folsom, CA, USA). Helium was used as a carrier gas. The initial temperature of the first column was 70°C for 30 sec and the subsequent temperature program was a heating rate of 5°C min^{-1} until 260°C was reached and held isothermally for a further 5 min. The initial temperature of the second column was 85°C for 30 sec and a 5°C min^{-1} heating rate was used until 275°C was reached and held isothermally for a further 5 min.

The composition of volatiles obtained from dried *T. cadmeum* leaves and/or flowers using DTD and HD is presented in Table 1. The yield of essential oil from *T. cadmeum* using HD was 1.35%. Sixteen main compounds have been labelled. The number of components identified in samples from flowers and leaves using DTD and leaves and flowers together using HD was 43, 46, and 42, respectively. The thirty-three components identified were common to both the DTD and HD techniques.

1) The University of Gaziantep, Engineering Faculty, Food Engineering Department, 27310 Gaziantep, Turkey; 2) The University of Pamukkale, Faculty of Science & Arts, Biology Department, P.O. 286, 20017, Denizli, Turkey; 3) The University of York, School of Chemistry, Heslington, YO10 5DD, York, U.K.; 4) The University of Pamukkale, Faculty of Science & Arts, Chemistry Department, P.O. 286, 20017, Denizli, Turkey, fax: +90 258 2953593, e-mail: mozel@pau.edu.tr.
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TABLE 1. Percentage Compositions of *T. cadmeum* Volatile Components Isolated Using Hydrodistillation (HD) and Direct Thermal Desorption (DTD) Techniques

Compound[a]	RI[b]	Volatiles of leaves of *T. cadmeum* using DTD		Volatiles of flowers of *T. cadmeum* using DTD		Volatiles of leaves and flowers of *T. cadmeum* using HD	
		%[c]	RSD[d]	%	RSD	%	RSD
3-Methylbutanal	641	0.87	4.69	0.85	5.43	–[e]	–
2-Methylbutanol	739	0.61	3.85	0.64	3.71	–	–
Hexanal	801	0.16	6.28	0.28	6.83	–	–
2,3-Butanediol	806	0.08	6.81	0.78	3.89	–	–
α-Thujene	938	2.45	4.83	1.02	4.43	–	–
α-Pinene	939	3.85	5.74	2.49	3.65	1.28	4.44
Camphene	953	1.95	2.99	0.64	4.51	0.45	6.89
Benzaldehyde	960	–	–	0.07	7.09	0.07	6.71
β-Pinene	981	2.19	6.75	3.49	5.17	1.82	3.84
2-Carene	994	–	–	6.18	4.40	3.40	5.53
α-Phellandrene	1006	1.68	5.84	1.35	6.08	0.38	7.62
3-Carene	1009	1.23	6.34	0.14	6.71	0.34	3.26
p-Cymene	1027	11.93	8.02	9.33	5.52	7.16	4.68
Eucalyptol	1030	26.97	6.04	0.82	4.47	12.01	4.71
γ-Terpinene	1074	1.37	4.95	15.29	6.02	3.57	4.18
Terpinolene	1088	0.76	6.83	0.29	8.17	0.53	7.09
Linalool	1100	0.05	7.11	0.58	3.73	0.14	4.67
Nonanal	1104	0.13	8.47	–	–	0.06	6.20
Phenylethyl alcohol	1118	0.09	7.41	0.10	8.84	–	–
α-Campholenal	1125	0.47	6.53	0.09	6.26	0.25	5.43
L-Pinocarveol	1139	0.16	7.19	0.17	5.19	0.50	4.47
Camphor	1139	1.15	4.46	0.50	6.71	1.95	5.79
cis-Verbenol	1140	–	–	0.39	3.84	0.66	4.75
Sabina ketone	1156	0.10	7.73	0.22	6.33	0.12	6.94
Borneol	1162	1.33	6.28	–	–	0.11	8.13
Terpinen-4-ol	1179	0.39	5.51	0.52	4.85	1.20	3.85
p-Cymen-8-ol	1183	0.34	7.35	0.36	6.59	0.52	5.47
Myrtenal	1194	0.05	8.80	0.07	8.81	0.12	6.19
α-Terpineol	1195	0.70	6.06	1.15	7.62	0.60	6.50
Carveol	1197	0.61	7.61	0.44	5.15	0.55	3.88
Verbenone	1204	3.66	6.33	2.38	6.64	5.67	6.57
Nerol	1232	0.21	5.06	–	–	0.16	5.94
Piperitol	1233	2.15	4.84	0.47	3.89	0.66	3.48
Cumin alcohol	1251	1.62	6.15	1.53	5.83	0.68	4.41
Bornyl acetate	1283	1.85	4.18	0.35	6.18	2.08	5.76
Ascaridol	1300	22.42	4.29	39.77	5.13	45.96	6.15
Thymol	1290	0.15	5.87	0.10	4.31	0.05	5.68
2-Caren-10-al	1292	–	–	0.11	6.15	0.08	6.10
α-Terpinenyl acetate	1348	0.09	7.03	0.06	8.17	0.05	7.08
Eugenol	1364	0.23	5.60	0.29	5.66	0.14	6.24
Ledene oxide	1440	0.05	7.76	1.08	4.29	0.29	6.83
α-Gurjunene	1443	–	–	0.12	7.16	0.08	7.98
Germacrene D	1482	0.05	8.13	0.06	6.43	0.17	4.43
α-Curcumene	1553	–	–	0.12	8.66	0.25	5.35
Caryophyllene oxide	1573	0.10	4.94	0.08	4.91	0.09	7.23
Spathulenol	1619	0.22	6.94	0.10	7.88	0.29	7.08
Dibutyl phthalate	1965	0.19	8.30	0.16	8.57	–	–
Eicosane	2000	0.27	7.71	0.29	6.37	0.24	5.93
Hexadecenoic acid	2380	0.05	8.91	0.12	7.28	0.07	8.97
Unknown	–	5.02	6.12	4.56	5.96	5.19	5.05

RI: retention indices; [a]As identified by GC×GC-TOF/MS software; names according to NIST mass spectral library, and by comparing their Kovats retention indices; [b]Kovats retention indices (column: DB 5); [c]Percentage of each component is calculated as peak area of analyte divided by summarized peak area of total ion chromatogram (In the case of multiple identification, the areas of the peaks that belong to one analyte were combined to find the total area for this particular analyte); [d]The relative standard deviations (RSD) for four (n = 4) experiments; [e]Not detected or percentage of the component is lower than 0.05%.

Phenylethyl alcohol (0.09–0.10%), dibutyl phthalate (0.16–0.19%), and the first five components listed in Table 1 (3.75–4.17% in total) isolated by the DTD technique were not found using HD. Esteban et al. [8] also reported that recovery of both low volatile and thermally labile compounds was better using thermal desorption compared with hydrodistillation. Almost the same number of components (at concentrations above 0.05%) was found in the DTD and HD volatile fractions.

As can be seen from Table 1, the major components in the leaf volatiles of *T. cadmeum* were eucalyptol (26.97%), ascaridol (22.42%), and *p*-cymene (11.93%). In the flowers, the main compounds were ascaridol (39.77%), γ-terpinene (15.29%), and *p*-cymene (9.33%). As leaves and flowers were used together in HD, the volatile compounds show similar trends. The main difference between the samples of leaves and flowers is the percentage amounts of eucalyptol (26.97 and 0.82%) and γ-terpinene (1.37 and 15.29%), respectively. In addition, using DTD, benzaldehyde, 2-carene, *cis*-verbenol, 2-caren-10-al, α-gurjunene, and α-curcumene were not detected in the leaves of *T. cadmeum* but were found in flowers. Conversely, nonanal, borneol, and nerol were not detected in flowers but were detected in leaves. According to the literature, there have been no previous publications concerning the volatile (essential oil) composition of *T. cadmeum.* Caliskan et al. [10] only looked at the crystal structures of eight lactones, two coumarins, and two flavonoid derivatives from *T. cadmeum*. There are not many *Tanacetum* species whose chemical compositions have not been explored. Looking at *Tanacetum* species reported in previous studies [1–6], there are considerable differences in the essential oil compositions. Kitchlu et al., [4] found 39 compounds in *T. gracile*, 16 of which agree with this study. They found lavandulol (21.5%), 1,8-cineole (15.2%), and α-pinene (11.2%) to be the main components. Ozer et al. [3], found camphor (54.4%) to be the main compound from *T. sorbifolium* (total 29 compounds). Nori-Shargh et al. [6] found camphor (53.5–59.1%), camphene (10.9–14.8%), and 1,8-cineole (7.8–10.1%) as the major constituents in the volatile oil of the flowers and leaves of *T. polycephelum*. Differences in the quality or quantity of the composition of essential oils may be due to collection time, differing chemotypes, drying conditions, mode of distillation and/or extraction, and geographic or climatic factors.

It can be concluded that hydrodistillation is still a very well known and widely used technique in the essential oil industry. However, it has longer processing times. On an industrial scale, hydrodistillation would still be the main method used in actual extraction of essential oil, but DTD could be used to check which components are being missed out in this process very quickly and easily without the need for time-consuming, expensive sample preparation.

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