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Comparison of the volatiles of *Daphne pontica* L. and *D. oleoides* Schreber subsp. *oleoides* isolated by hydro- and microdistillation methods

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Abstract: Aerial parts of *Daphne pontica* were collected from Ilgaz-Çankırı, and *D. oleoides* subsp. *oleoides* was collected from 2 different localities (Ayrancı-Karaman and Ilgaz-Çankırı) in Turkey. The samples were subjected to hydrodistillation and microdistillation. The resulting volatile samples were analyzed both by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS), respectively. The main components for *D. pontica* were identified as hexahydrofarnesyl acetone (8.6%), carvacrol (8.5%), dihydroedulane II (4.7%), (*E*)-geranyl acetone (4.6%), and thymol (4.5%), while nonacosane (42.5% and 27.2%), hexadecanoic acid (24.4% and 20.0%), phytol (12.3%), and carvacrol (5.0%) were identified as the main components of *D. oleoides* subsp. *oleoides* obtained by hydrodistillation. Carvacrol (12.0%), thymol (7.7%), dihydroactinidiolide (7.2%), bicyclosesquiphellandrene (5.5%), and (*Z*)-3-hexenal (4.1%) were the major components in *D. pontica*, while carvacrol (27.2% and 25.4%), nonacosane (24.6%), (*Z*)-3-hexenal (18.5% and 2.5%), decane (7.4%), hexahydrofarnesyl acetone (7.4% and 2.2%), hexanal (6.6% and 1.5%), heptacosane (6.1%), nonanal (5.6% and 1.9%), thymol (5.1% and 2.3%), and phytol (5.0%) were identified in the *D. oleoides* subsp. *oleoides* isolated by microdistillation, respectively. In addition, the volatile components were evaluated for their ability to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals using a bioautographic thin layer chromatography (TLC) method, and the samples showed activity comparable with that of the tested standards, vitamins C and E.

Key words: *Daphne* sp., essential oil, volatiles, hydrodistillation, microdistillation, gas chromatography, gas chromatography-mass spectrometry, antioxidant activity

1. Introduction

The genus *Daphne* (Thymelaeaceae) is distributed throughout Anatolia and is represented by 7 species in Turkey: *D. mezereum* L., *D. pontica* L., *D. glomerata* Lam., *D. sericea* Vahl., *D. oleoides* Schreber, *D. gnidioides* Jaub. & Spach, and *D. mucronata* Royle (1). These species are known to be quite hazardous to humans due to their potentially toxic resins. In fact, some poisoning cases in children who consumed the fruits of these species have been reported. Thus, the *Daphne* species growing in Turkey, and especially its fruits, should not be consumed (2). Use of the genus *Daphne* is also mentioned in Turkish traditional folk medicine according to our previous investigations and reports (2–6). *D. oleoides* Screber is used against rheumatism/edema, lumbago, abscess, and malaria and in wound healing. It is also used for the treatment of lamed animals. Different local names were given to this species such as ezenteri, çoban süpürgesi, çıtlak, havadana, and develikotu (2–5). *D. pontica* L. is known as tasma, kurtbağı, sırımağu, and sırımbağı and is used against diarrhea in traditional medicine (2,6). In field surveys *Daphne* species were known as mezeliyon, yabani taflan, yırcık, eğircik çalısı, iğircik, emicik, and havaza, without distinguishing between species, and were used for their diuretic, diaphoretic, laxative, and wound healing properties. Their use against abdominal or miscellaneous pains and indigestion as well as in veterinary care has been documented in Anatolia (2,7–9).

In vivo studies revealed that *D. oleoides* possessed anti-inflammatory and antinociceptive activities; however, the results were statistically not significant (10). *D. oleoides* subsp. *oleoides* showed potent inhibitory activity against

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the biosynthesis of inflammatory cytokines interleukin-1 (IL-1α, IL-1β) and tumor necrosis factor (TNF-α) (11,12). Phytochemical studies reported that *D. oleoides* contains terpenoids, sterols, coumarins, lignans, flavonoids, and aromatic compounds, among others (2,13–20).

The roots of *D. pontica* were reported to show significant anti-inflammatory activity on carrageenan-induced, PGE_2-induced hind paw edema and 12-*O*-tetradecanoyl-13-acetate–induced mouse ear edema in mice (21). Flavonoids, coumarins, and sterols were isolated from *D. pontica* in previous phytochemical studies (22–24).

In the present study the aerial parts of *D. oleoides* subsp. *oleoides* and *D. pontica* collected from Ilgaz-Çankırı and Ayrancı-Karaman (Turkey) were investigated for their essential oil, volatile compounds, and antioxidant activity for the first time. The plant materials were subjected to hydrodistillation as well as microdistillation for the isolation of the essential oils and volatiles. The samples were analyzed both by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) to determine their chemical compositions. The radical scavenging activities of the samples were evaluated by TLC-bioautography.

2. Materials and methods

2.1. Plant materials

D. oleoides Schreber subsp. *oleoides* was collected from Yukarıkıramanoğlu village, Ayrancı, Karaman, in July 1999 (sample 1) and Ilgaz Mountain, Çankırı, in July 2003 (sample 2). *D. pontica* L. was collected from around the Doruk Hotel (2070 m) Ilgaz Mountain, Çankırı (Turkey) in June 1999 and kept frozen until use. Voucher specimens were identified by Dr AM Gençler Özkan and Prof Dr E Yeşilada and deposited at the Gazi University Herbarium, Faculty of Pharmacy.

2.2. Hydrodistillation

The air dried aerial parts of plant materials were each hydrodistilled separately for 3 h using a Clevenger-type apparatus according to the method recommended in the European Pharmacopoeia (25). As the amount of essential oil was relatively low, the volatiles were trapped in *n*-hexane and subsequently dried over anhydrous Na_2SO_4 and stored at 4 °C until further use.

2.3. Microdistillation

The microdistillation technique was applied as in previous studies (26,27). Briefly, the crushed aerial plant parts (approximately 100 mg) were placed in the sample vial with distilled water. NaCl, distilled water, and *n*-hexane were added into the collecting vial to trap the volatile components. After distillation was completed the immiscible solvents containing the volatiles were stored at 4 °C until further use. All of the isolated volatile samples were analyzed both by GC and GC-MS, simultaneously.

2.4. Analysis of the essential oils

2.4.1. GC-MS analysis

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

2.4.2. GC analysis

The GC analysis was carried out using an Agilent 6890N GC system. The FID detector temperature was set to 300 °C. To obtain the same elution order as GC-MS, simultaneous auto-injection was performed on a duplicate of the same column where the same operational conditions were applied. Relative percentages (%) of the separated compounds were calculated from FID chromatograms. The results are shown in Tables 1 and 2.

2.5. Identification and characterization of components

Characterization of the essential oil components was carried out by comparing their relative retention times with those of authentic samples or by comparing their relative retention index (RRI) to a series of *n*-alkanes (C_9-C_{20}). Computer matching against a commercial (Wiley GC/MS Library, Adams Library, MassFinder 2.1 Library) (28,29) and in-house Baser Library of Essential Oil Constituents built up through the genuine compounds and components of known oils, as well as MS literature data (30–32), was used for identification (Table 1).

2.6. Antioxidant evaluation (TLC bioautographic DPPH radical scavenging activity)

According to the methods of Kumarasamy et al. (33) and Sarker et al. (34), 1 mL of stock solution was prepared from each test sample and control. From each of these 5 μL was spotted separately on to the TLC plate. After development, the TLC plate was air dried for complete solvent evaporation, and 0.2% (w/v) DPPH in MeOH was sprayed onto the duplicate TLC plate after application and development of the samples.

3. Results and discussion

Due to the relatively low essential oil yields (<0.01%), the volatiles were trapped in *n*-hexane and were isolated by conventional hydrodistillation from samples 1 and 2 of *D. oleoides* subsp. *oleoides* and from *D. pontica*. For comparison, the same plant materials were subjected to microdistillation. The volatiles obtained were further analyzed simultaneously by GC and GC-MS (35).

As shown in Table 1, the volatiles obtained by hydrodistillation from *D. pontica* comprised 51

Table 1. Chemical composition of the volatiles of *Daphne* species obtained by different distillation methods.

RRI	Compound	1	2	3	4	5	6
1000	Decane	-	-	-	-	-	7.4
1093	Hexanal	-	1.2	-	1.5	-	6.6
1203	Limonene	-	tr	-	0.2	-	3.9
1213	1,8-Cineole	-	tr	-	0.6	-	2.3
1225	(*Z*)-3-Hexenal	2.7	4.1	-	2.5	-	18.5
1280	*p*-Cymene	0.2	tr	-	-	-	2.4
1294	1,2,4-Trimethyl benzene	-	0.1	-		-	1.8
1348	6-Methyl-5-hepten-2-one	0.7	-	-	1.2	-	1.5
1360	Hexanol	-	-	-	1.0	-	0.8
1391	(*Z*)-3-Hexenol	2.0	3.4	-	-	-	1.0
1400	Nonanal	1.4	2.7	-	1.9	-	5.6
1400	Tetradecane	0.2	-	-	-	-	-
1487	Citronellal	-	1.8	-	-	-	-
1496	2-Ethyl hexanol	-	-	-	0.8	-	-
1500	Pentadecane	0.3	-	-	-	-	-
1505	Dihydroedulane II *	4.7	3.4	-	-	-	-
1506	Decanal	-	-	0.4	1.9	-	-
1532	Camphor	-	1.3	-	-	-	-
1541	Benzaldehyde	0.6	-	-	-	-	-
1553	Linalool	0.4	1.0	-	1.3	-	-
1562	Octanol	0.5	1.2	-	1.3	-	1.2
1591	Bornyl acetate	-	1.6	-	-	-	-
1600	Hexadecane	0.4	-	-	-	0.5	-
1602	6-Methyl-3,5-heptadien-2-one	0.5	2.5	-	1.2	-	-
1611	Terpinen-4-ol	0.5	0.3	-	0.5	-	-
1612	β-Caryophyllene	0.5	0.3	-	0.5	-	-
1616	Hotrienol	0.2	-	-	-	-	-
1617	Undecanal	-	-	0.5	1.0	-	-
1638	β-Cyclocitral	0.4	0.8	-	-	-	-
1639	Cadina-3,5-diene	-	3.1	-	-	-	-
1654	1-Hexadecene	1.1	-	-	-	-	-

Table 1. Continued.

RRI	Compound	1	2	3	4	5	6
1688	10-Methyl-2-undecanone	0.8	-	-	-	-	-
1700	Heptadecane	0.6	-	-	-	0.6	-
1706	a-Terpineol	-	-	-	0.3	-	-
1719	Borneol	-		-	0.1	-	-
1722	Bicyclosesquiphellandrene	-	5.5	-	-	-	-
1722	Dodecanal	-	-	-	0.3	-	-
1729	Zonarene	-	1.7	-	-	-	-
1741	β-Bisabolene	0.1	-	-	-	-	-
1763	Naphthalene	0.1	-	-	-	-	-
1766	Decanol	-	-	0.2	-	-	-
1772	Citronellol	-	1.9	-	-	-	-
1773	d-Cadinene	0.4	-	-	-	-	-
1776	γ-Cadinene	0.1	-	-	-	-	-
1798	Methyl salicylate	0.3	-	-	-	-	-
1800	Octadecane	0.3	-	-	-	0.7	-
1830	Tridecanal	-	-	1.1	-	-	-
1838	(*E*)-β-Damascenone	0.8	-	tr	-	-	-
1849	Calamenene	1.3	1.2		-	-	-
1868	(*E*)-Geranyl acetone	4.6	3.1	0.7	0.5	0.5	4.0
1890	Carvacryl acetate	-	-	0.2	-	-	-
1900	Nonadecane	0.3	-	-	-	1.1	-
1933	Tetradecanal	0.2	0.5	-	0.7	0.5	-
1958	(*E*)- β-Ionone	2.9	3.1	0.6	0.6	0.1	-
1973	Dodecanol	0.6	-	0.7	0.2	-	-
2000	Eicosane	-	-	-	-	0.8	-
2008	Caryophyllene oxide	-	-	0.8	-	-	-
2009	*trans*-β-Ionone-5,6-epoxide	0.7	3.0	-	-	-	-
2030	Methyl eugenol	-	-	0.4	1.8	-	-
2036	2-Pentadecanone	0.2	-	-	-	-	-
2050	(*E*)-Nerolidol	0.9	-	-	-	-	-
2100	Heneicosane	0.8	-	-	-	1.1	-

Table 1. Continued.

RRI	Compound	1	2	3	4	5	6
2131	Hexahydrofarnesyl acetone	8.6	3.6	3.8	2.2	2.3	7.4
2148	(*Z*)-3-Hexen-1-yl benzoate	1.1	-	1.3	1.3	0.3	-
2179	3,4-Dimethyl-5-pentylidene-2(5H)-furanone	1.2	1.3	0.5	0.9	-	-
2179	Tetradecanol	-	-	0.8	-	-	-
2198	Thymol	4.5	7.7	1.0	5.1	-	2.3
2200	3,4-Dimethyl-5-pentyl-5H-furan-2-one	0.4	1.3	-	0.5	-	-
2200	Docosane	-	-	-	-	1.7	-
2226	Methyl hexadecanoate	-	-	-	-	0.6	-
2239	Carvacrol	8.5	12.0	5.0	25.4	1.6	27.2
2255	a-Cadinol	-	0.7	-	-	-	-
2300	Tricosane	1.8	0.8	1.3	0.5	2.0	-
2380	Dihydroactinidiolide	0.5	7.2	-	3.0	-	-
2384	Farnesyl acetone	2.7	1.0	1.2	1.7	2.1	-
2400	Tetracosane	0.6	-	0.5	-	0.9	-
2500	Pentacosane	1.2	0.5	1.6	-	3.7	-
2503	Dodecanoic acid	-	-	-	-	1.0	-
2512	Benzophenone	0.2	-	-	-	-	-
2607	1-Octadecanol	-	-	2.4	-	-	-
2622	Phytol	-	-	1.2	-	12.3	5.0
2670	Tetradecanoic acid	-	-	4.0	-	5.3	-
2700	Heptacosane	1.1	1.4	0.8	6.1	7.2	-
2800	Octacosane	-	-	-	-	0.7	-
2900	Nonacosane	3.5	1.0	42.5	24.6	27.2	-
2931	Hexadecanoic acid			20.0		24.4	-
	Total	**69.2**	**87.3**	**93.5**	**93.2**	**99.2**	**98.9**

1: *D. pontica* = hydrodistillation
2: *D. pontica* = microdistillation
3: *D. oleoides* subsp. *oleoides* (plant sample 1) = hydrodistillation
4: *D. oleoides* subsp. *oleoides* (plant sample 1) = microdistillation
5: *D. oleoides* subsp. *oleoides* (plant sample 2) = hydrodistillation
6: *D. oleoides* subsp. *oleoides* (plant sample 2) = microdistillation
RRI: relative retention indices calculated against *n*-alkanes (C_9–C_{20})
%: calculated from FID data
tr: trace (<0.1%)
*: correct isomer not identified

compounds representing 69.2% of the total. The major components of the samples obtained by hydrodistillation were: hexahydrofarnesyl acetone (8.6%), carvacrol (8.5%), dihydroedulane II (4.7%), (*E*)-geranyl acetone (4.6%), and thymol (4.5%). In comparison, only 39 compounds were detected from the volatiles obtained by the microdistillation method, where detected compounds constituted 87.3%. Carvacrol (12.0%), thymol (7.7%), dihydroactinidiolide (7.2%), bicyclosesquiphellandrene (5.5%), and (*Z*)-3-hexenal (4.1%) were the major components of this fraction.

The other species investigated in this study, *D. oleoides* subsp. *oleoides*, was collected from 2 different localities (a southern province, Karaman, and a northern province, Çankırı) in Turkey. After GC and GC-MS analyses of the hydrodistilled oils 27 (93.5%) and 25 (99.2%) compounds were identified from the Karaman (sample 1) and Çankırı (sample 2) specimens, respectively. As seen in Table 1, both samples were rich in nonacosane, hexadecanoic acid, and tetradecanoic acid. In addition, carvacrol (5.0%) was the major component in the Karaman sample, while phytol (12.3%) and heptacosane (7.2%) were the major constituents in samples from Çankırı. While 34 (93.2%) compounds were detected in the volatiles obtained by microdistillation from the Karaman sample, only 17 compounds (98.9%) were found the Çankırı sample. The major compounds of sample 1 were carvacrol (25.4%), nonacosane (24.6%), heptacosane (6.1%), and thymol (5.1%) by microdistillation; sample 2 contained carvacrol (27.2%), (*Z*)-3-hexenal (18.5%), decane (7.4%), hexahydrofarnesyl acetone (7.4%), hexanal (6.6%), nonanal (5.6%), phytol (5.0%), and (*E*)-geranyl acetone (4.0%). Qualitative and quantitative GC and GC-MS comparison of hydrodistillation and microdistillation results revealed that composition of the volatiles showed considerable variation, which might be due to the different isolation techniques used, especially in the cases of hydrocarbon and oxygenated monoterpenes and fatty acids and their esters, as seen in Table 2.

Hydrodistillation is the common and classical methodology for isolating volatiles from aromatic plants; however, the major drawbacks are the need for a large amount of samples (generally, 10–100 g of dried plant material) and the tedious work up and intensive time involved (36). When compared with conventional hydrodistillation, the microdistillation method offers fast and practical handling and comparatively good qualitative and quantitative results, making it valuable for volatile analysis. As a result, microdistillation is a handy method for the fast isolation of volatile compounds, even for amounts of plant or organic material measured in milligrams, rendering it quite useful for phytochemical research and analyses (26).

Previous studies of the volatiles of different *Daphne* species were restricted to a traditional Chinese remedy, which compared the composition of processed and unprocessed *D. genkwa* materials by GC-MS (37,38).

For the activity evaluation experiments in the present work, 1 mL standardized volumes of the *n*-hexane–trapped samples (1–6) obtained both by hydrodistillation and microdistillation were applied to TLC plates, which were subsequently treated with DPPH solution after evaporation of the solvent to faded yellow spots. *D. oleoides*

Table 2. Relative percentage (%) distribution of volatiles in *Daphne* samples.

Compound groups	1	2	3	4	5	6
Monoterpene hydrocarbons	0.3	-	-	0.2	-	6.4
Oxygenated monoterpenes	27.6	36.1	7.8	38.2	2.1	36.2
Sesquiterpene hydrocarbons	3.5	13.5	-	0.5	-	-
Oxygenated sesquiterpenes	5.2	2.0	2.1	1.8	2.1	-
Diterpenes	-	-	1.3	-	12.4	5.0
Fatty acids+esters	-	-	25.7	-	31.6	-
Others	63.4	48.4	63.1	59.3	51.8	52.4

1: *D. pontica* = hydrodistillation
2: *D. pontica* = microdistillation
3: *D. oleoides* subsp. *oleoides* (plant sample 1) = hydrodistillation
4: *D. oleoides* subsp. *oleoides* (plant sample 1) = microdistillation
5: *D. oleoides* subsp. *oleoides* (plant sample 2) = hydrodistillation
6: *D. oleoides* subsp. *oleoides* (plant sample 2) = microdistillation

subsp. *oleoides* hydrodistillation samples, in particular, suggested radical scavenging activity and antioxidant properties when compared with the standard antioxidants.

There are only a few studies on the antioxidant activity of *Daphne* species, and almost all these studies were conducted on *D. gnidium* L. (39,40). The ethnobotanical uses (2–9) as well as biological and pharmacological activities of *Daphne* species (2,10–12,21,41,42) are interesting and promising despite the toxic constituents of the species.

Due to the biological, pharmacological, and toxicological properties of *Daphne* species, more comprehensive in vitro and in vivo biological activity studies along with phytochemical studies are needed in order to provide more insight in to this interesting genus, not only in Turkey but globally.

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