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Comparative essential oil analysis of *Geranium sylvaticum* extracted by hydrodistillation and microwave distillation

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The essential oil of *Geranium sylvaticum* L. (Geraniaceae) was isolated by hydrodistillation (HD) and microwave distillation (MD). The composition of the volatile oils was characterized by GC-FID and GC-MS. A total of 73 and 70 compounds were identified, constituting over 90.3% and 98.7% of oil composition of *G. sylvaticum*, respectively. Sesquiterpene hydrocarbons were shown to be the main group of constituents (HD: 31.7% and MD: 39.8%). The major component of the oils of *G. sylvaticum* was γ-muurolene (HD: 10.9% and MD: 19.6%). The comparative study showed that the amount of total volatiles (98.7%) and the major constituent (39.8%) were better in the MD of *G. sylvaticum*.

Key Words: *Geranium sylvaticum*, Geraniaceae, hydrodistillation, microwave distillation, essential oil, GC-FID, GC-MS

Introduction

The genus *Geranium* L. (Geraniaceae) is represented by over 300 species of annual, biennial, and perennial taxa growing mainly in cool-temperate regions of the world.[1] This genus is the largest one of the family *Geraniaceae*. Many taxa of the genus are well known ornamental plants because of their attractive flowers/leaves and there are 46 native taxa, 13 of which are endemic to Turkey.[1] A number of species from this genus are cultivated as medicinal plants, and extracts from the roots of some *Geranium* spp. are used in traditional medicine, tanning, and dyeing.[2] Recently, many articles about the composition, biological effects, and use in medicine,

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food-flavoring, perfumery, and cosmetics of the essential oils of *Geranium* have been published.[3−12] However, no published study has previously reported the essential oil composition of *G. sylvaticum* growing in Turkey. The species has been used in the north Anatolian villages for feeding animals along with other alpine herbaceous plants. It has no history of medicinal use in folk medicine in Anatolia.

Essential oils in plants are complex mixtures of volatile substances present in low concentrations. Several extraction processes have been used in order to obtain essential oils of the plants. Recently, a microwave distillation has been developed for extracting volatile products.[13−16]

The purpose of the present investigation was to extend our knowledge of the essential oil of *G. sylvaticum* (Table 1) and compare the essential oil composition of *G. sylvaticum* extracted by microwave distillation and hydrodistillation. We make appropriate comparisons in term of extraction yields, times, and essential oil composition.

Table 1. Identified components in the essential oils of *G. sylvaticum*$^{a-c}$ extracted by HD and MD.

Compounds	HD % Area	MD % Area	Exp. RI	Lit. RI
Monoterpene hydrocarbons				
Limonene^{c}	0.4	0.2	1030	1030
$\Delta-3-$Carene	0.4	-	1032	1031
(Z)-β-Ocimene	0.4	-	1040	1037
Terpinolene	0.5	0.3	1089	1089
Oxygenated monoterpenes				
Linalool^{c}	10.1	3.2	1100	1097
α-Terpineol^{c}	2.2	1.0	1190	1189
β-Cyclocitral	0.3	0.2	1217	1217
Nerol	0.2	0.1	1232	1230
Geraniol^{c}	0.7	0.9	1255	1253
Sesquiterpene hydrocarbons				
α-Cubebene	0.4	0.2	1351	1351
α-Copaene	0.3	0.1	1376	1377
β-Bourbonene	1.1	0.6	1386	1388
β-Cubebene	-	0.3	1387	1388
β-Elemene	1.9	0.8	1390	1391
$(E)-$Caryophyllene	5.7	6.7	1418	1419
β-Copaene	0.5	0.4	1430	1432
γ-Elemene	0.3	-	1434	1437
α-Guaiene	0.2	-	1438	1440
cis-Muurola-3,5-diene	0.3	0.1	1446	1450
α-Humulene	1.2	1.7	1454	1455
Farnesane	0.7	0.8	1463	1462
$\gamma-$Muurolene	10.9	19.6	1479	1480
Germacrene D	0.5	0.7	1484	1485

Table 1. Contunied.

Compounds	HD % Area	MD % Area	Exp. RI	Lit. RI
α-Alaskene	0.6	2.0	1496	1498
α-Muurolene	0.5	0.2	1499	1500
E,E-α-Farnesene	2.9	3.5	1504	1506
β-Bisabolene	1.7	1.1	1507	1506
γ-Cadinene	0.3	0.2	1516	1514
β-Curcumene	1.6	0.5	1518	1516
Germacrene B	0.1	0.3	1558	1561
Oxygenated sesquiterpenes				
(*E*)-Nerolidol	0.3	0.3	1565	1563
Caryophyllene oxide	0.7	0.9	1580	1583
Viridiflorol	0.3	0.3	1590	1593
Epi-α-Muurolol	0.4	0.5	1641	1642
Cubenol	0.1	0.2	1645	1647
α-Cadinol	0.7	0.7	1653	1654
Intermedeol	0.1	0.2	1669	1667
Oxygenated Diterpene				
Phytol	0.1	0.2	1944	1943
Terpene related compounds				
Geranyl acetone	0.5	0.1	1455	1455
β-Ionone	0.6	-	1486	1489
Hexahydro farnesylacetone	1.2	2.5	1846	1847
Farnesyl acetone	0.2	0.8	1917	1919
Aldehydes				
Benzaldehyde	0.5	2.7	958	960
2*E*,4*E*-Heptadienal	-	0.2	1013	1012
Benzene acetaldehyde	6.9	4.7	1045	1042
o-Tolualdehyde	-	0.2	1064	1068
Nonanal	4.7	5.0	1103	1101
2*E*,6*Z*-Nonadienal	0.7	0.4	1157	1155
2*E*-Nonenal	1.0	0.8	1164	1162
Decanal	5.5	4.3	1205	1202
Undecanal	0.2	0.2	1309	1307
2*E*,4*E*-Decadienal	0.2	0.5	1318	1317
Dodecanal	0.8	1.3	1409	1410
Tetradecanal	0.4	0.4	1613	1613
Pentadecanal	2.2	2.6	1713	1713
Hexadecanal	0.1	0.2	1814	1816

Table 1. Contunied.

Compounds	HD % Area	MD % Area	Exp. RI	Lit. RI
Hydrocarbons				
Decane[c]	2.4	1.1	1000	1000
Dodecane[c]	0.2	0.3	1198	1200
Tetradecane[c]	0.3	-	1400	1400
Heptadecane[c]	0.2	0.1	1697	1700
Nonadecane[c]	0.1	0.6	1899	1900
Eicosane[c]	0.2	0.6	1997	2000
Heneicosane[c]	1.1	2.7	2101	2100
Docosane[c]	0.4	0.9	2197	2200
Tricosane[c]	3.9	8.8	2296	2300
Tetracosane[c]	1.7	0.9	2399	2400
Pentacosane[c]	1.2	2.2	2497	2500
Others				
1-Octen-3-ol	0.5	0.7	981	979
2-Pentyl furan	1.4	0.7	992	993
1,2,4-Trimethyl benzene	0.2	-	1025	1026
2-Undecanone	0.3	0.1	1296	1294
2-Methylene-cyclopentanol	0.4	0.2	1362	1359
E-Asarone	0.2	0.2	1377	1376
Benzyl benzoate	0.1	0.2	1764	1760
Hexadecanol	0.1	0.4	1880	1876
Palmitic acid	-	1.8	1981	1980
Octadecanol	0.1	0.3	2080	2078

[a] RI calculated from retention times relative to that of n-alkanes (C_5-C_{32}) on the non-polar HP-5 column.

[b] Percentages obtained by FID peak-area normalization.

[c] Identified using authentic samples.

Materials and methods

Plant material

G. sylvaticum L. was collected in Şalpazarı (at heights of ∼1710 m), Trabzon, in the northeastern part of Turkey in July, 2008. The plant was authenticated by Prof. S. Terzioğlu.[1] A voucher specimen was deposited in the Herbarium of the Faculty of Forestry, KATO (KATO: 16512), Karadeniz Technical University, Turkey.

Hydrodistillation apparatus and procedure

The fresh plant materials (∼100 g, each) were ground into small pieces and subjected to hydrodistillation (HD) using a Clevenger-type apparatus with a cooling bath (-15 °C) system (3 h) (yield (v/w): 0.08%). The obtained oils were extracted with HPLC grade n-hexane (0.5 mL) and dried over anhydrous sodium sulfate and stored at -5 °C in a sealed brown vial.

Microwave distillation apparatus and procedure

Microwave distillation (MD) was performed at atmospheric pressure with a Milestone DryDIST microwave apparatus using a fixed power of 650 W for 30 min. Temperature was monitored by an external infrared sensor. The fresh plant materials (∼100 g, each) were ground into small pieces, then placed in a round bottom flask (2 L) with 50 mL water, and subjected to microwave distillation (MD) using a Clevenger-type apparatus with cooling bath (-15 °C) system (30 min) (yield (v/w): 0.06%). The obtained oils were extracted with HPLC grade n-hexane (0.5 mL) and dried over anhydrous sodium sulfate and stored at -5 °C in a sealed brown vial.

Gas chromatography (GC)

The capillary GC-FID analysis was performed using an Agilent-5973 Network System, equipped with a FID (supplied with air and hydrogen of high purity) and a split inlet. The chromatographic column used for the analysis was an HP-5 capillary column (30 m × 0.32 mm i.d., film thickness 0.25 μm). Helium was used as carrier gas, at a flow rate of 1 mL/min. The injections were performed in splitless mode at 230 °C. Then 2 μL of essential oil solution in hexane (HPLC grade) was injected and analyzed with the column held initially at 60 °C for 2 min and then increased to 240 °C with a 3 °C/min heating ramp. The identity of each compound was supported by comparing their retention indices (RI) with published values.[16−26] The sample was analyzed twice and the percentage composition of oil was computed from the GC peak areas without using correction factors.

Gas chromatography-mass spectrometry (GC/MS)

GC-MS analysis was performed using an Agilent-5973 Network System. A mass spectrometer with an ion trap detector in full scan mode under electron impact ionization (70 eV) was used. The chromatographic column used for the analysis was an HP-5 capillary column (30 m × 0.32 mm i.d., film thickness 0.25 μm). Helium was used as carrier gas, at a flow rate of 1 mL/min. The injections were performed in splitless mode at 230 °C. Then 2 μL of essential oil solution in hexane (HPLC grade) was injected and analyzed with the column held initially at 60 °C for 2 min and then increased to 240 °C with a 3 °C/min heating ramp.

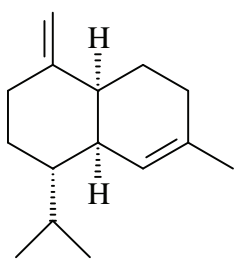
Identification of constituents

Retention indices of all the components were determined by the Kovats method using n-alkanes (C_5-C_{32}) as standards. The constituents of the oils were identified by comparison of their mass spectra with those of mass spectral libraries (NIST and Wiley), authentic compounds (limonene, linalool, α-terpineol, geraniol, decane,

dodecane, tetradecane, heptadecane, nonadecane, eicosane, heneicosane, docosane, tricosane, tetracosane, and pentacosane), and with data published in the literature.[17−23]

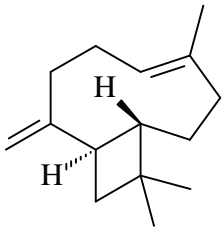
Results and discussion

The chemical composition of the essential oils of *G. sylvaticum* extracted by HD and MD is presented in Table 1. A total of 78 different components (73 HD vs. 70 MD) were identified by GC-FID and GC-MS with the HP-5 column (Table 1). The higher number of compounds extracted by HD (73 components) compared to MD (70 components) is probably related to the possible degradation of products by oxidation or hydrolysis, because of a longer extraction time (3 h for HD vs. 30 min for MD) and a greater quantity of water (2 L for HD vs. 100 mL for MD). The MD oils could be distinguished from the HD oils by their richness in sesquiterpene hydrocarbons (39.8% MD and 31.7% HD), aldehydes (23.5% MD and 23.2% HD), and hydrocarbons (18.2% MD and 11.7% HD). The HD oils could be differentiated from the MD oils by their greater richness in oxygenated monoterpenes (13.2% HD and 5.4% MD). The main constituents (Figure) in HD and MD were γ-muurolene (10.9% HD and 19.6% MD), linalool (10.1% HD and 3.2% MD), $(E)-$caryophyllene (5.7% HD and 6.7% MD), *E,E*-α-farnesene (2.9% HD and 3.5% MD), benzene acetaldehyde (6.9% HD and 4.7% MD), nonanal (4.7% HD and 5.0% MD), decanal (5.5% HD and 4.3% MD), and tricosane (3.9% HD and 8.8% MD).



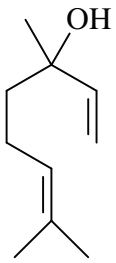
γ-Muurolene

19.6% by MD and 10.9% by HD



(E)-Caryophyllene

6.7% by MD and 5.7% by HD



Linalool

3.2% by MD and 10.1% by HD

Figure. Main components in the essential oils of *G. sylvaticum.*

The chemical class distributions of the volatile constituents of *G. sylvaticum* are summarized in Table 2. The compounds were separated into 4 classes: terpenoids (monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, oxygenated diterpene, and terpene related compounds), aldehydes, hydrocarbons, and others (Table 2). The major constituents were sesquiterpene hydrocarbons (39.8% MD and 31.7% HD), aldehydes (23.5% MD and 23.2% HD) and hydrocarbons (18.2% MD and 11.7% HD) in the oils of *G. sylvaticum.* The numbers of the identified terpenoids in HD and MD of *G. sylvaticum* were 37 and 34, respectively. Comparison of *Geranium* volatiles with those mentioned in the literature[3−8] indicates that identified compounds are in most cases similar in the essential oil.

Extraction time of 30 min (MD) provides yields comparable to those obtained after 3 h by means of HD. As noted previously,[13−16] the reduced cost of extraction is clearly advantageous for the MD method in terms of

time and energy.[13] In comparison with HD, MD offers important advantages, such as similar yields, cleanliness of the process, shorter extraction times, and substantial savings of energy.

Table 2. The chemical class distribution in the essential oils of *G. sylvaticum.*

Constituents	HD		MD	
	% Area	NC[a]	% Area	NC[a]
Terpenoids				
Monoterpene hydrocarbons	1.7	4	0.5	2
Oxygenated monoterpenes	13.2	5	5.4	5
Sesquiterpene hydrocarbons	31.7	20	39.8	19
Oxygenated sesquiterpenes	2.6	7	3.1	7
Oxygenated diterpenes	0.1	1	0.2	1
Terpene related compounds	2.5	4	3.4	3
Aldehydes	23.2	12	23.5	14
Hydrocarbons	11.7	11	18.2	10
Others	3.3	9	4.6	9
Total	90.3	73	98.7	70

[a]NC: Number of compounds

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