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Chemical Constituents and Antimicrobial Activity of the Essential Oil from *Vicia dadianorum* Extracted by Hydro and Microwave Distillations

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Abstract: The aim of this research was to investigate the influence of extraction methods on yield and chemical composition of the essential oil of *Vicia dadianorum* Somm. & Lev. The volatiles of *V. dadianorum* have been isolated by hydro and microwave distillations (HD and MD). The compositions of the essential oils were characterized by GC-FID and GC-MS. A total of seventy-six and fifty-six compounds were identified, constituting over 90.9%, and 80.1% of oil composition of *V. dadianorum*, respectively. Sesquiterpene hydrocarbons were shown to be the main group of volatiles (HD: 26.2% and MD: 15.9%). The major terpene constituent of the essential oils of *V. dadianorum* was γ-elemene (HD, 13.7% and MD, 8.4%). Comparative study showed that the amount of total volatiles (90.9%) and the major constituent (26.2%) were found to be better in HD of *V. dadianorum.* The antimicrobial activity of the isolated essential oils of the plant was also investigated, and it showed moderate antimicrobial and antifungal activities against *Staphylococcus aureus, Enterococcus faecalis*, *Bacillus cereus*, *Mycobacterium smegmatis* and *Candida albicans*.

Keywords: *Vicia dadianorum,* hydrodistillation; microwave distillation; essential oil, antimicrobial activity; GC-MS.

1. Introduction

The genus *Vicia* L. (Leguminosae) is represented with 61 native species including 87 intraspecific taxa, 5 of them is endemics in Turkey [1-3]. *Vicia* is a medium sized genus but it is economically considerable genus because of the two early domesticated plants: *V. faba* and *V. ervilia* [4]. This genus includes some food crops and forage plants such as *V. sativa* cultivated in many countries including Turkey [5]. *V. dadianorum* Somm. & Lev. is a perennial herb with a creeping rootstock and it is grown

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in subalpine meadows. It is a Euxine element and mainly distributed in NE Anatolia [1].

Essential oils in plant are complex volatile mixtures exist at low concentrations. Before analyzing the oils, they have to be extracted from the plant. Several extraction processes have been used in order to obtain the high yield of components [6]. The effect of different distillation methods on oil content and composition of aromatic plants have been previously mentioned [7-9]. Recently, a microwave distillation has been developed for extracting volatile products [10-14].

V. faba has been investigated for volatiles [15-16]. However, no published study has previously reported on the essential oil composition of *V. dadianorum* grown in Turkey. The present study was designed to analyze the chemical composition and compare the essential oil contents of *V. dadianorum* extracted by hydrodistillation and a microwave distillation as well as to evaluate their antimicrobial activity.

2. Materials and Methods

2.1. Plant Material

V. dadianorum was collected in Yağmurdere valley, Gümüşhane-Turkey (at heights of ~2020 m) in the northeastern part of Turkey in May, 2010. The plant was authenticated by Prof. S. Terzioğlu [1-4]. Voucher specimen was deposited in the Herbarium of the Faculty of Forestry, KATO (KATO: 12171), Karadeniz Technical University, Turkey.

2.2. Hydrodistillation Apparatus and Procedure

The fresh plant material (150 g) were grounded into small pieces and submitted to hydrodistillation (HD) using a Clevenger-type apparatus with cooling bath (-15 ^{o}C) system (4h) (yield (v/w): 0.035%). The obtained oil was extracted with HPLC grade n-hexane (0.5 mL) and dried over anhydrous sodium sulphate and stored at -5 ºC in a sealed brown vial.

2.3. Microwave Distillation Apparatus and Procedure

Microwave distillation (MD) was performed at atmospheric pressure with a Milestone DryDIST microwave apparatus using a fixed power of 750 W for 40 min. Temperature was monitored by an external Infrared (IR) sensor. The fresh plant material (150 g) were grounded into small pieces, then placed in a round bottom flask (2 L) with 50 ml water and submitted to microwave distillation (MD) using a Clevenger-type apparatus with cooling bath (-15 ^{o}C) system (yield (v/w): 0.055%). The obtained oil was extracted with HPLC grade n-hexane (0.5 mL) and dried over anhydrous sodium sulphate and stored at -5 ºC in a sealed brown vial.

2.4. Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-FID and GC-MS analyses were done as described previously [14].

2.5. Identification of Constituents

The components of the oils were identified by comparison of their mass spectra with those of a computer library or with authentic compounds (α-pinene, α-terpineol, linalool, pentadecane, heptadecane, heneicosane, docosane, tricosane, tetracosane, and pentacosane) and confirmed by comparison of their retention indices, either with those of authentic compounds or with data published in the literature [17-28].

2.6. Antimicrobial Activity Assessment

All test microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: *Escherichia coli* ATCC 25922, *Yersinia pseudotuberculosis* ATCC 911, *Pseudomonas auroginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* 709 ROMA, *Mycobacterium smegmatis* ATCC607 and *Candida albicans* ATCC 60193. All the essential oils were dissolved in hexane to prepare chemicals stock solution of 9.000-21.100 µg /400 µL.

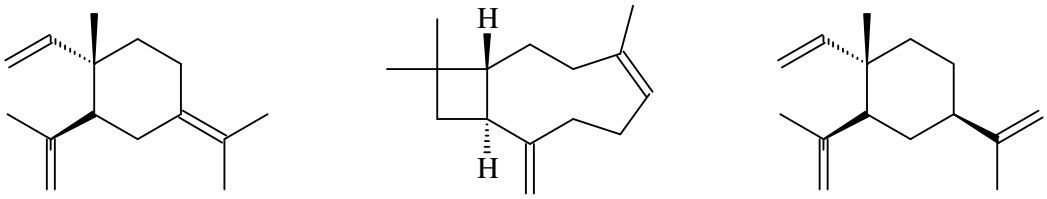
2.7. Agar Well Diffusion Method

Simple susceptibility screening test using agar-well diffusion method [29] as adapted earlier [30] was used. Each bacterium was suspended in Mueller Hinton (MH) (Difco, Detroit, MI) broth. The yeast like fungi was suspended in Yeast extracts broth. Then the microorganisms were diluted approximately 10^6 colony forming unit (cfu) per mL. For yeast like fungi, Sabouraud Dextrose Agar (SDA) (Difco, Detriot, MI) were used. Brain Heart Infusion Agar (BHI) (Difco, Detriot, MI) was used for *M. smegmatis*. They were "flood-inoculated" onto the surface of MH and SD agars and then dried. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 40 µL of the extract substances were delivered into the wells. The plates were incubated for 18 h at 35°C. The *M. smegmatis* was grown for 3 days on BHI agar plates at 35 °C [31]. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. Ampicillin (10 µg), Streptomisin (10 µg) and fluconazole (5 µg) were standard drugs. Hexane was used as solvent control. The results are shown in table 2.

3. Results and Discussion

The volatile oils obtained after hydrodistillation and microwave distillation of the *V. dadianorum* gave an average yields (v/w) of 0.035% and 0.055%, respectively. In total, GC-MS analyzes allowed the identification of 86 volatile compounds [17-28] (76, HD and 56, MD), accounting for 90.0% and 80.1% of the detected GC peak areas, respectively. The list of the identified volatile constituents as well as their grouping into nine classes, namely monoterpene hydrocarbons, oxygenated monoterpens, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, terpene related compounds, aldehydes, esters, hydrocarbons and others with the ratios is given in table 1. The higher number of compounds extracted by HD (76 components) compared to MD (56 components) is probably related to the possible degradation of products by oxidation or hydrolysis, because a longer extraction time (3 h for HD and 30 min for MD) and a greater quantity of water (2 L for HD and 50 ml for MD) used.

Sesquiterpene hydrocarbones were found as the major group of compounds in *V. dadianorum*, constituted 26.2% in HD and 15.9% in MD of the oils. Among them, γ-elemene (13.7% HD and 8.4% MD), *(Z)*-caryophyllene (2.6% HD and 1.5% MD), and β-elemene (2.6% HD and 1.1% MD) were identified as the main components (Figure 1).



γ-Elemene, 13.7% by HD and 8.4% by MD

(Z)-Caryophyllene, 2.6% by HD and 1.5% by MD

β-Elemene, 2.6% by HD and 1.1% by MD

Figure 1. Main sesquiterpene components in the essential oils of *V. dadianorum*

Linalool (6.5%) was the major compound of the oxygenated monoterpenes (1.6% HD and 1.1% MD). *V. dadianorum* oils were characterized by high content of aldehydes (17.4% HD and 19.8% MD), esters (10.0% HD and 19.2% MD), and terpene related compounds (9.8% HD and 8.4% MD). Among the aldehydes, (2*E*,4*E*)-decadienal (4.5% HD and 6.0% MD), nonanal (3.1% HD and 2.1% MD), (2*E*)-nonen-1-al (2.9% HD and 1.9% MD) were found to be the main constituents. Furthermore, hexahydro farnecylacetone (4.7% HD and 5.1% MD) was determined as the major constituent of the terpene related compounds. Monoterpene hydrocarbons were determined only in the oil of HD. The MD oil could be distinguished from the HD oil by their richness in aldehydes (17.4% HD and 19.8% MD) and hydrocarbons (10.0% MD and 19.2% HD). The HD oil could be differentiated from the MD oil by their greater richness in sesquiterpene hydrocarbones (26.2% HD and 15.9% MD), oxygenated monoterpenes (2.7% HD and 1.7% MD), and monoterpene hydrocarbones (1.4% HD and 0% MD). The numbers of the identified terpenoids in the oils of *V. dadianorum* were 38 (HD) and 16 (MD) compounds. The analysis of variance showed that the distillation method had a significant effect on the oil content of *V. dadianorum.* The highest oil yield was obtained by microwave distillation method. This may be due to fact that in microwave distillation, type and situation of plant material, mode of charging, and grade of insulation are more important than other distillation method. This result is in agreement with the previous work about the effect of distillation methods on oil contents of volatiles [7-9]. But, comparative study showed that the amount of total volatiles (90.9%) and the major constituent (26.2%) were found to be better in HD of *V. dadianorum.* Therefore, hydro-distillation could be recommended for the essential oil extraction of *V. dadianorum*.

Our results appeared to be quite different from previously reported data on the chemical composition of *Vicia* oil since it was devoid of esters, alcohols and oxygenated compounds [15]. The volatile odour chemical of the flowers of the *V. faba* has been identified as (*E*)-β-ocimene with only trace of other monoterpens [15-16]. Generally, the comparison of our data with the literature showed that the main constituents of chemical composition of the investigated *V. dadianorum* oils were sesquiterpene hydrocarbons and markedly different from known *V. faba* [15-16]. The difference of the composition of the oils could be attributed to the geographical source and the specific climate there.

The antimicrobial activity for the essential oils of *V. dadianorum* was tested *in vitro* using the agar-well diffusion method [29-31] with the microorganisms as seen in Table 2. The essential oils showed moderate antibacterial activity against Gram-positive bacteria *S. aureus*, *E. faecalis*, *B. cereus* 702 Roma, *M. smegmatis*, and pathogenic fungi (*C. albicans*).

Table 1. Identified components in the oils of *V. dadianorum* extracted by HD and MD.

Compounds	Area[a] (%) HD	Area[a] (%) MD	Ex. RI[b]	Lit. RI
Monoterpene hydrocarbons				
α-Pinene[c]	0.4	-	940	939
δ-3-Carene	0.8	-	1031	1031
(E)-*β*-Ocimene	0.2	-	1051	1050
Oxygenated monoterpenes				
Linalool[c]	1.6	1.1	1100	1097
α-Terpineol[c]	0.7	0.2	1189	1189
β-Cyclocitral	0.4	0.4	1217	1217
Sesquiterpene hydrocarbons				
α-Copaene	0.2	0.2	1379	1377
(E)-*β*-Damascenone	0.5	0.3	1382	1385
β-Elemene	2.6	1.1	1391	1391
β-Longipinene	0.2	-	1397	1401
(Z)-Caryophyllene	2.6	1.5	1410	1409
(E)-Caryophyllene	0.3	-	1419	1419
β-Duprezianene	-	0.2	1420	1423
γ-Elemene	13.7	8.4	1436	1437
cis-Prenyl limonene	0.4	0.3	1445	1446
(E)-*β*-Farnesene	1.1	0.7	1454	1457
α-Acoradiene	0.1	-	1468	1466
γ-Muurolene	0.7	0.9	1480	1480
β-Selinene	0.2	0.3	1490	1490
(E)- *β*-Bisabolene	0.3	0.3	1502	1506
β-Sesquiphellandrene	1.7	1.0	1520	1523
δ-Cadinene	0.2	-	1522	1523
Zonarene	0.3	-	1531	1530
Germacrene-B	1.1	0.7	1561	1561
Oxygenated sesquiterpenes				
Iso-italicene epoxide	0.3	-	1514	1515
(E)-Nerolidol	0.2	0.1	1561	1563
Caryophyllene oxide	0.6	0.4	1583	1583
Epi-*α*-cadinol	0.2	-	1639	1640
Epi-*α*-muurolol	0.2	-	1642	1642
Selina-3,11-dien-6-*α*-ol	0.3	-	1647	1644
Juniper camphor	1.6	-	1697	1700
(E,E)-Farnesol	-	0.2	1727	1725
Ledenol	0.9	0.4	1759	1761
Terpene related compounds				
α-Ionene	0.4	-	1256	1256
Dihydroedulan-I	1.9	0.8	1288	1289
1,1,6-Trimethyl-1,2-dihydronaphthalene	0.4	0.1	1351	1354
Geranyl acetone	0.4	0.3	1453	1455
(E)-*β*-Ionone	1.7	1.7	1488	1489
Hexahydro farnesylacetone	4.7	5.1	1846	1847
Farnesyl acetone	0.3	0.4	1916	1919
Aldehydes				
(2*E*,4*E*)-Heptadienal	-	0.6	1014	1015

Benzene acetaldehyde	0.3	1.0	1042	1042
(2*E*)-Octenal	0.5	0.7	1061	1062
Nonanal	3.1	2.2	1101	1101
(2*E*,6*Z*)-Nonadienal	1.5	1.7	1155	1155
(2*E*)-Nonen-1-al	2.9	1.9	1162	1162
Decanal	0.9	0.4	1204	1202
(2*E*,4*E*)-Nonadienal	-	0.4	1214	1212
(2*E*)-Decenal	1.7	1.6	1264	1264
(2*E*,4*Z*)-Decadienal	0.8	1.9	1291	1293
Undecanal	0.2	-	1304	1307
(2*E*,4*E*)-Decadienal	4.5	6.0	1316	1317
3-Dodecen-1-al	0.2	0.3	1362	1359
Dodecanal	0.5	0.5	1408	1409
Tridecanal	-	0.3	1507	1510
Tetradecanal	0.3	0.3	1612	1613
Esters				
(*Z*)-3-Hexenyl isovalerate	0.3	0.1	1235	1239
Hexyl isovalerate	0.3	-	1242	1244
(*Z*)-3-Hexenyl tiglate	1.6	0.8	1323	1322
Hexyl tiglate	0.2	-	1330	1333
(*E*)-2-Hexenyl tiglate	0.4	0.2	1338	1338
Benzyl tiglate	0.3	-	1495	1498
Methyl 8-(2-furyl) octanoate	0.1	0.1	1625	1627
Methyl tetradecanoate	-	0.2	1721	1724
Methyl hexadecanoate	0.6	0.8	1922	1922
Ethyl hexadecanoate	0.3	-	1992	1993
Methyl linoleate	0.6	0.5	2094	2096
Methyl linolenate	2.2	-	2102	2101
Methyl octadecanoate	-	0.1	2123	2125
Ethyl linoleate	0.2	0.3	2145	2146
Ethyl linoleolate	0.4	0.5	2158	2161
Hydrocarbons				
Pentadecane[c]	-	0.4	1499	1500
Heptadecane[c]	0.6	0.1	1700	1700
1-Octadecene	0.2	0.2	1789	1790
Heneicosane[c]	2.5	3.1	2099	2100
Docosane[c]	0.9	1.2	2198	2200
Tricosane[c]	2.7	8.3	2302	2300
Tetracosane[c]	1.1	1.0	2400	2400
Pentacosane[c]	2.0	4.9	2500	2500
Others				
1-Octen-3-ol	1.3	1.7	979	979
3-Octanone	0.6	0.8	982	984
3-Octanol	-	1.1	988	991
2-Pentyl furan	9.0	2.8	991	993
1-Octanol	0.4	0.6	1070	1068
(6*Z*)-Nonen-1-ol	-	0.5	1174	1171
Hexadecanoic acid	0.3	2.9	1984	1980

			NC[d] (HD)	NC[d] (MD)
Monoterpene hydrocarbons	1.4	-	3	-
Oxygenated monoterpenes	2.7	1.7	3	3
Sesquiterpene hydrocarbons	26.2	15.9	17	3
Oxygenated sesquiterpenes	4.3	1.1	8	4

Terpene related compounds	9.8	8.4	7	6
Aldehydes	17.4	19.8	13	15
Esters	7.5	3.6	13	10
Hydrocarbons	10.0	19.2	7	8
Others	11.6	10.4	5	7
Total	90.9	80.1	76	56

[a]Percentages obtained by FID peak-area normalization. [b]RI calculated from retention times relative to that of n-alkanes (C_6-C_{32}) on the non-polar HP-5 column. [c]Identified by authentic samples. [d] NC: Number of compounds

Table 2. Screening result for antimicrobial activity of the essential oils from *V. dadianorum*.

Samples	Stock (µg/ 400 µL)	Microorganisms and inhibition zone (mm)							
		Ec	*Yp*	*Pa*	*Sa*	*Ef*	*Bc*	*Ms*	*Ca*
V. dadianorum (HD)	9.000	-	-	-	8	8	10	30	nt
V. dadianorum (MD)	21.100	-	-	-	10	-	10	30	20
Ampicillin	10	10	10	18	35	10	15	-	-
Streptomycin	10	-	-	-	-	-	-	35	
Fluconazole	5	-	-	-	-	-	-	-	25

Ec: E. coli, *Yp*: *Y. pseudotuberculosis*, *Pa*: *P. aeruginosa*, *Sa*: *S. aureus*, *Ef*: *E. faecalis*, *Bc*: *B. cereus* 702 Roma, *Ms*: *M. smegmatis*, *Ca*: *C. albicans*, (-): no activity, nt: not tested.

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