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Characterization of Lipids and Fatty Acid Methyl Ester Contents in Leaves and Roots of *Crocus vallicola*

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The chemical composition of the fatty acids methyl esters (FAMEs) and other lipids in leaves and roots of *Crocus vallicola* were analyzed by gas chromatography-mass spectrometry (GC-MS). In this work, twenty-eight compounds, including 22 FAMEs, 1 aldehyde, 3 hydrocarbons (substitute alkane and alkene), 2 alcohols in the leaves and twenty-one compounds (17 FAMEs, 1 anhydride, 1 substitute alcohol, 1 ketone, 1 substitute amide) in the roots were identified by GC-MS from *C. vallicola*. The FAMEs in leaves and roots were highly similar; only the amounts of the esters were different.

Key Words: *Crocus vallicola*, leaves, roots, FAMEs

Introduction

Although many of the lipids and FAMEs of various plants have been extensively investigated to obtain volatile components[1−14], the need still remains for unstudied plants. *C. vallicola* is a plant belonging to the Iridaceae family and is widely distributed in northern Turkey[15]. Prior to the initiation of this work, no study appears to have been carried out on lipids and FAMEs in the the leaves and roots of *C. vallicola*. The objective of this study was to identify and quantify the major volatiles so as to see the differences in leaves and roots of *C. vallicola*.

There are various methods for identifying the fatty acid composition of plants. Among them GC-MS is one of the most commonly used techniques to determine the composition of the volatile oil of *C. vallicola*. Not all organic compounds are suitable for direct GC-MS analysis due to their involatile nature. Many important biological compounds, such as fatty acids, flavonoids, alkaloids, carbohydrates, amino acids and terpenoids are polar, and have limited volatility. Two main approaches adopted for the examination of analytes do not seem to satisfy the normal criteria of volatility for GC-MS. Either they are degraded under controlled conditions by pyrolysis to give characteristic volatile fragments or they are derived into related compounds that are suitable for gas chromatography. In this study, the MeOH extracts of leaves and roots of *C. vallicola* were hydrolyzed and methylated using usual procedures and analyzed with GC-MS.

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Experimental

Materials: Samples of *C. vallicola* were collected from the northern region of Turkey in October 1997. All plants, leaves and roots were separately cut into small pieces before lipid extraction. The wet leaves (0.650 kg) and roots (0.319 kg) were extracted with cold MeOH (1 l, 2x-24 h, each). The total MeOH extracts were filtered, and the filtrates were concentrated on a rotary evaporator at 30°C to obtain the crude mixtures (leaves, 1.52 g; roots, 0.850 g).

Hydrolysis of Lipids: The lipid samples (∼100 mg) were refluxed with 0.1 M potassium hydroxide solution in 95% ethanol (2 ml) for 1 hour. The solutions were cooled, water was then added (5 ml) and the aqueous mixture was neutralized with 0.5 M HCl and extracted with hexane-diethyl ether (1:1 v/v; 3x, 5 ml). The organic layer was separated and washed with water (10 ml), dried over anhydrous Na_2SO_4 and filtered for each sample. The free fatty acids were recovered after solvent evaporation in vacuo for both leaves and roots.

The Preparation of Methyl Esters of Fatty Acids: The lipid samples (∼75 mg) were dissolved in toluene (1 ml) in a test tube fitted with a condenser, and to this was then added H_2SO_4 in methanol (2 ml, 1%). The mixtures were left overnight in a stoppered tube at 50°C then sodium chloride solution (5 ml, 5%) was added and the required esters were extracted with hexane (2x, 5 ml), then the organic layer was separated using Pasteur pipettes for both samples. The hexane layers were washed with potassium bicarbonate solution (4 ml, 2%) and dried over anhydrous Na_2SO_4 and filtered. The organic solvent was removed under reduced pressure on a rotary evaporator to give FAMEs and other lipids.

GC-MS Conditions: GC-MS analysis was performed in a Fisons MD800 mass (quadrupole) - GC8000 series instrument equipped with a flame ionization detector. A silica column (30 m x 0.25 mm I.D.) coated with OV1 was utilized. The initial temperature was 40°C for 4 min after injection, then increased to 280°C (8°C/ min) with a final hold at 280 °C for 20 min. The injector and detector temperature were maintained at 270°C and 250°C, respectively. Helium was used as the carrier gas at a flow-rate of 0.8 ml/min.

Results and Discussion

In this report, the volatile fatty acid methyl ester and other lipid components in leaves and roots of *C. vallicola* were compared. The plant leaves and roots were extracted with MeOH separately. Crude extracts were saponified in aqueous methanolic potassium hydroxide solution and then methylated with methanol in 1% H_2SO_4. Therefore, in order to identify the hydrolyzed volatile chemical constituents of the leaves and roots of *C. vallicola*, a capillary gas chromatographic-mass spectrometric method was employed for profiling total fatty acids methyl esters and other lipid contents of *C. vallicola* and various types of compounds such as hydrocarbons, alcohols, ketones, anhydride and FAMEs were identified[1−11] (Table).

Total run time of the leaves and the roots were 30 min and 34 min, respectively. A total of 49 FAMEs and other lipids were identified by relative retention times compared to those of known FAMEs using a comprehensive databank of NBS and Wiley library for identification, 28 and 27 of which were from leaves and roots, respectively (Table). In the Table, the compounds are listed in order of elution on a capillary column. The mass fragmentation patterns of compounds are also listed in the Table. Confirmation of identities by mass spectral structure elucidation revealed saturated, unsaturated and branched-chain FAMEs. The presence of alcohols, ketones, anhydrides, hydrocarbons and aldehydes were also confirmed.

Table FAMEs and Lipid Components in Leaves and Roots of *Crocus vallicola* and Mass Spectral Data Thereof (Obtained from GC/MS, Run 70 eV, r.t.:22.66 is solvent, 31.70% in leaves and 37.73% in roots)

No	Compounds	RT	Leaves %	Roots %	M^+	Mass Spectral Data, m/z (rel. intensity)
1	Hexanal	5.38	0.60	-	100=$[C_6H_{12}O]^+$	100(3), 82(12), 72(20), 67(13), 57(52), 56(85), 44(100), 43(55) 41(75).
2	1-Propoxy-2-propanol	6.48	19.56	16.93	118=$[C_6H_{14}O_2]^+$	118(2), 74(5), 73(40), 63(12), 61(43), 45(100), 44(11), 43(52) 42 (5).
3	Methyl octanoate	12.41	1.15	-	158=$[C_9H_{18}O_2]^+$	127(8), 115(10), 101(9), 87(45), 74(100), 69(9), 57(18), 43(32), 41(22).
4	Methyl 8-oxooctanoate	15.44	0.79	1.45	172=$[C_9H_{16}O_3]^+$	172(3), 141(28), 129(43), 97(72), 87(100), 74(62), 69(75), 55(68), 43(62), 41(73).
5	Methyl decanoate	15.51	0.97	-	186=$[C_{11}H_{22}O_2]^+$	186(3), 155(7), 143(12), 129(8), 101(10), 87(55), 74(100), 55(24), 43(32).
6	Methyl cyclopentaneundecanoate	16.58	1.08	-	286=$[C_{17}H_{32}O_2]^+$	199(2), 144(10), 124(7), 101(30), 87(51), 74(100), 69(23), 55(75), 43(49), 41(50).
7	Methyl 9-Oxononanoate	16.94	9.16	5.92	186=$[C_{10}H_{18}O_3]^+$	186(2), 158(6), 155(12), 143(28), 115(13), 111(43), 87(65), 74(100), 69(28), 55(74), 43(48).
8	1-(1-Ethoxy ethoxy)octane	17.85	0.79	-	202=$[C_{12}H_{26}O_2]^+$	187(7), 155(5), 123(4), 95(13), 81(7), 75(100), 71(12), 43(24), 41(10).
9	Methyl dodecanoate	18.17	0.94	0.83	214=$[C_{13}H_{26}O_2]^+$	214(3), 183(4), 171(8), 143(12), 129(10), 87(60), 74(100), 57(12), 55(23), 43(32), 41(28).
10	Dimethyl nonanedioate	18.41	1.03	-	216=$[C_{11}H_{20}O_4]^+$	185(28), 151(70), 124(22), 111(51), 97(33), 83(64), 74(78), 55(100), 43(48), 41(49).
11	Methyl 7-hexadecenoate	18.90	0.53	-	268=$[C_{17}H_{32}O_2]^+$	166(5), 138(23), 110(13), 98(50), 83(52), 69(70), 55(100), 43(52), 41(100).
12	8,8-Dimethoxy-2,6-dimethyl-2-octanol	19.07	4.55	-	218=$[C_{12}H_{26}O_3]^+$	218(3), 201(8), 169(4), 137(7), 109(6), 95(7), 76(5), 75(100), 71(12), 41(8).
13	Methyl 8-(2-furyl)octanoate	19.41	0.43	-	224=$[C_{13}H_{20}O_3]^+$	224(8), 193(5), 137(3), 123(14), 95(72), 81(100), 55(22), 41(22).
14	Methyl tetradecanoate	20.52	1.09	2.75	242=$[C_{15}H_{30}O_2]^+$	242(4), 211(5), 199(13), 185(5), 143(18), 129(5), 101(6), 87(70), 74(100), 55(30), 43(38).
15	Methyl pentadecanoate	21.59	0.54	0.78	256=$[C_{16}H_{32}O_2]^+$	256(3), 225(3), 213(12), 185(5), 157(6), 143(12), 129(10), 101(8), 87(67), 74(100), 55(32), 43(39).
16	6,10,14-trimethyl-2-pentadecanone	21.80	-	0.62	268=$[C_{18}H_{36}O]^+$	251(2), 179(2), 165(3), 137(4), 124(10), 109(22), 95(22), 85(25), 71(40), 58(70), 53(2), 43(100).
17	Methyl 9-hexadecenoate	22.41	0.83	-	268=$[C_{17}H_{32}O_2]^+$	268(4), 236(12), 194(10), 152(13), 110(18), 96(40), 83(45), 74(52), 69(60), 55(100), 41(70).
18	Methyl 7-hexadecenoate	22.43	-	0.86	268=$[C_{17}H_{32}O_2]^+$	268(2), 236(12), 194(10), 137(10), 123(13), 110(12), 96(48), 83(50), 74(55), 69(63), 67(38), 59(22), 55(100), 43(48).
19	Methyl hexadecanoate	22.63	6.82	7.53	270=$[C_{17}H_{34}O_2]^+$	270(5), 239(4), 227(10), 199(6), 185(6), 157(3), 143(18), 129(12), 101(6), 87(65), 74(100), 57(16), 55(22), 43(31).
20	14-Methyl methylhexadecanoate	23.34	0.61	-	284=$[C_{18}H_{36}O_2]^+$	284(6), 255(3), 241(10), 205(3), 185(12), 143(18), 111(10), 97(18), 87(12), 74(100), 69(22), 57(28), 55(45), 43(42).
21	Methyl heptadecanoate	23.59	0.51	0.52	284=$[C_{18}H_{36}O_2]^+$	284(3), 253(4), 241(9), 199(8), 185(10), 143(12), 101(9), 87(72), 74(100), 55(32), 43(38).
22	2-Hydroxy, methylhexadecanoate	23.84	0.95	-	286=$[C_{17}H_{34}O_3]^+$	286(3), 254(2), 241(3), 227(30), 201(10), 145(10), 125(14), 111(42), 97(92), 83(95), 69(87), 55(100), 43(98), 41(73).
23	Methyl octadecanoate	24.19	0.31	4.48	298=$[C_{19}H_{38}O_2]^+$	298(3), 255(12), 213(3), 199(7), 185(3), 143(14), 129(3), 101(6), 87(78), 74(100), 55(23), 43(28).
24	Methyl 10-octadecenoate	24.30	3.79	4.98	296=$[C_{19}H_{36}O_2]^+$	296(3), 264(8), 222(6), 180(10), 166(3), 152(5), 123(10), 97(42), 83(44), 69(52), 55(100), 43(32), 41(63).
25	Methyl 9-octadecenoate	24.35	-	2.44	296=$[C_{19}H_{36}O_2]^+$	296(2), 265(8), 264(15), 222(9), 207(4), 180(7), 138(8), 111(18), 97(42), 83(43), 69(53), 55(100), 41(52).
26	16-Methyl, methylheptadecanoate	24.50	4.64	-	298=$[C_{19}H_{38}O_2]^+$	298(10), 267(3), 255(13), 241(3), 213(4), 199(10), 185(6), 157(2), 143(18), 129(8), 101(6), 87(70), 74(100), 55(26).
27	1,1-dimethoxy 9-octadecene	25.38	0.37	-	312=$[C_{20}H_{40}O_2]^+$	213(32), 149(26), 127(86), 95(20), 71(100), 55(18), 43(12), 41(25).
28	Methyl (11R, 12R, 13S)-12,13-epoxy-11-methoxy-9-octadecenoate	25.47	0.98	-	340=$[C_{20}H_{36}O_4]^+$	271(3), 239(14), 227(25), 201(10), 169(26), 163(14), 93(12), 85(43), 71(100), 45(32), 43(20).

Table Contunie

No	Compounds	RT	Leaves %	Roots %	M^+	Mass Spectral Data, m/z (rel. intensity)
29	Methyl-3-octyloxirane-octanoate	25.84	-	1.29	312=$[C_{19}H_{36}O_3]^+$	199(6), 181(4), 171(11), 155(36), 139(18), 127(20), 109(22), 97(32), 87(38), 83(40), 74(56), 69(56), 55(100), 41(63).
30	N-Methyl-N-4,4-methoxy-1-hexahydropyridyl-2-butyl acetamide	26.04	-	2.23	238=$[C_{13}H_{22}O_2N_2]^+$	238(2), 207(8), 199(10), 181(12), 167(25), 139(12), 121(24), 97(36), 95(50), 81(52), 69(54), 57(98), 55(100), 43(84).
31	Methyl eicosanoate	26.21	-	0.86	326=$[C_{21}H_{42}O_2]^+$	326(8), 283(8), 255(2), 241(3), 227(5), 185(5), 143(21), 126(8), 101(9), 97(12), 87(78), 74(100), 55(34), 43(42).
32	2-Dodecen-1-yl (-) succinic anhydride	26.43	-	3.55	266=$[C_{16}H_{26}O_3]^+$	223(3), 209(4), 181(6), 166(5), 151(6), 149(12), 137(22), 123(32), 109(38), 97(41), 83(55), 69(86), 55(94), 41(100).
33	8-Nitro-11-dodecanolide	27.56	0.64	-	243=$[C_{12}H_{21}O_4N]^+$	243(2), 227(16), 213(12), 165(11), 141(48), 115(32), 83(30), 71(100), 55(50), 41(32).
34	Methyl docosanoate	27.79	0.59	1.89	354=$[C_{23}H_{46}O_2]^+$	354(12), 311(10), 269(5), 255(6), 185(8), 143(23), 112(12), 87(82), 74(100), 69(22), 57(32), 43(48), 41(22).
35	Methyl tricosanoate	28.52	-	1.04	368=$[C_{24}H_{48}O_2]^+$	368(10), 325(6), 283(2), 269(5), 213(2), 199(10), 185(5), 143(26), 101(12), 97(14), 87(82), 74(100), 55(42), 43(54).
36	Methyl tetra-cosanoate	29.34	0.42	4.03	382=$[C_{25}H_{50}O_2]^+$	382(12), 339(9), 297(5), 283(5), 255(4), 341(3), 199(6), 185(5), 143(22), 97(14), 87(82), 74(100), 57(42), 43(58).
37	Methyl penta-cosanoate	30.20	-	0.81	396=$[C_{26}H_{50}O_2]^+$	396(11), 353(8), 311(4), 297(5), 255(5), 241(4), 199(8), 185(7), 143(22), 97(16), 87(80), 74(100), 57(45), 43(58).

The GC-MS analysis of leaves and roots of *C. vallicola* allowed the identification of 28 components in the leaves and 21 components in the roots. The leaf mixture is made up of 17 FAMEs (56%) as major constituents, 2 alcohols (40%), 3 hydrocarbons (3%) and 1 aldehyde (1%) as minor constituents. And the root mixture is made up of 17 FAMEs (64%) as major constituents, 1 anhydride (5%), 1 alcohol (26%), 1 amide (4%) and 1 ketone (1%) as minor constituents. The fatty acid composition of the leaves and roots of *C. vallicola* was very similar. The percentage of the identified FAMEs components in roots (64%) were relatively higher than leaves (56%) but the amount of methyl 9-oxononanoate (9.16%) and methyl dodecanoate (0.94%) in leaves was higher than in the roots (see Table and Figure). The major constituent of the leaves and roots was metyhl-9-oxononanoate with 9.16% and 5.92% , respectively, and the minor constituent was methyl octadecanoate, (0.31%) in leaves and methyl heptadecanoate (0.52%) in the roots of *C. vallicola*. The chain lengths of FAMEs were between C_9 to C_{25} in *C. vallicola*. Among the unsubstituted saturated and unsaturated fatty acid methyl ester, methyl, 10-octadecenoate (19:10) was the major component of esters in leaves (3.79%) and roots (4.98%). In the GC-mass spectrum, the proportions of saturated FAMEs were higher than unsaturated FAMEs. From the results of this investigation, it is obvious that eleven of the FAMEs and one alcohol in different ratios were the same in both the leaves and roots of *C. vallicola* (see Figure). Therefore, these results clearly indicate that both leaves and roots have different FAMEs components. They both have similar 11 FAMEs; however, leaves have 11 different FAMEs and roots have 6 different FAMEs. As for the amount of FAMEs, 56% total FAMEs was found in the leaves and 64% was found in the roots. The amount of similar FAMEs in leaves constitutes 34% of 56% and 51% of 64% in roots.

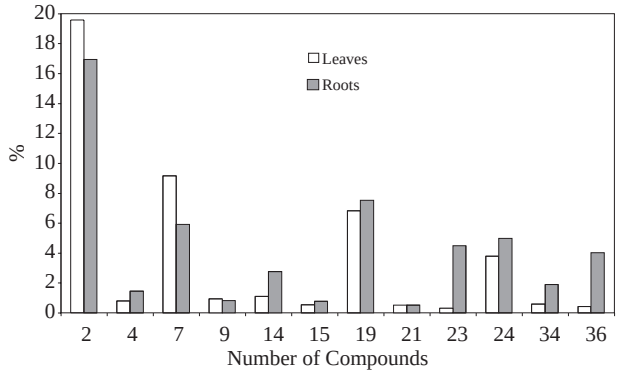

Figure The percentage of FAMEs and alcohol component in leaves and roots of C*rocus vallicola.*

This is the first fatty acid methyl esters and other lipids contents report in both the leaves and roots of *C. vallicola* studied by GC-MS.

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