Chemistry of Natural Compounds, Vol. 42, No. 6, 2006

FATTY ACID COMPOSITION OF *Viscum album* SUBSPECIES FROM TURKEY

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UDC 665.325

The lipophylic extracts of three Viscum album *subspecies growing on different host plants in Turkey were comparatively analyzed for derived methyl esters of their fatty acids by capillary gas chromatography-mass spectrometry (GC-MS). The sample of* V. album *ssp.* album *growing on apricot trees was remarkably rich in palmitic acid (11.47%). Arachidic acid was found in only ssp.* austriacum *and ssp.* abietis *samples. The unsaturated fatty acids, mainly oleic and linoleic acids, were identified in nine lipophylic extracts obtained from* V. album *samples belonging to ssp.* album. *The amounts of linoleic and oleic acid were the highest in the sample of ssp.* album *growing on apricot trees (12.18 and 9.19%, respectively).*

Key words: fatty acid, Loranthaceae, GC-MS, ssp. *album*, ssp. *abietis*, ssp. *austriacum*, Omega-6, Omega-3.

Viscum album L. (European mistletoe) (Loranthaceae), which is an evergreen, semiparasitic plant found on the branches of deciduous trees, is represented by three subspecies; namely ssp. *album*, ssp. *abietis* (Wiesb.) Abromeit, and ssp. *austriacum* (Wiesb.) Vollmann [1].

Since ancient times, European mistletoe has been used in the treatment of diabetes mellitus, convulsions, arthrosis, cancer, hypertension, and arteriosclerosis [2, 3].

Phytochemical studies on *V. album* have revealed the presence of some pharmacologically active substances including alkaloids, polysaccharides, phenylpropanes, lignans, lectins, viscotoxins, and flavonoids [4, 5].

Linoleic and α-linolenic acids, obtained from plant material, in the diet are the precursors in tissues of two families with opposing effects which are referred to as "essential fatty acids" (EFA): arachidonic acid (AA) and pentane (eicosopentaenoic acid: EPA) and hexaene (docosahexaenoic acid: DHA) acids. The role of EFA is crucial; without a source of AA or compounds which can be converted into AA, synthesis of prostaglandins (PGs) by a cyclooxygenase (COX) enzyme would be compromised, and this would seriously affect many normal metabolic processes [6].

The purpose of this research was to determine the fatty acid composition of lipophylic (leaves, stems, and twigs) extracts from eleven *V. album* samples belonging to three *V. album* subspecies growing in Turkey: ssp. *album*, ssp. *abietis*, and ssp. *austriacum*. This is the first report on the fatty acid content of lipophylic extracts prepared from leaves, stems, and twigs of *V. album* subspecies growing on different host plants in Turkey. Therefore, any comparison with previously published data was not possible.

In this study, lipophylic extracts were obtained from leaves, stems, and twigs of *V. album* samples. These parts of plants are known to be poor in fatty acids. On the other hand, a considerable content of sterols and aliphatic alcohols was noted in all samples. But many of these compounds could not be identified by GC-MS apparatus. Therefore, the sum (10.10–36.15%) of fatty acids of extracts is not close to 100%.

Among the eleven *V. album* samples, the highest oil yield was obtained from ssp. *album* leaves, twigs, and stems growing on pear tree (11.95%), and ssp. *austriacum* leaves, twigs, and stems growing on pine tree gave the lowest oil yield (1.32%).

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0009-3130/06/4206-0641

TABLE 1. Fatty Acid Composition of the Lipophylic Extracts of *V. album* Subspecies

Fatty acid identified	R, min	Relative composition, %*										
		ssp. *album*									ssp. *austriacum*	ssp. *abietis*
		AVA	AVW	PE	PC	RP	CO	CA	CV	PD	PN	AB
Saturates												
16:0	6.9	11.47±0.41	3.09	4.10	4.49	1.20	-	3.09	1.27±0.35	1.31±0.19	5.29±0.48	1.85
18:0	10.8	1.62	0.70	1.25	0.92	1.55	1.61	0.70	0.79	1.44±0.15	1.02	1.13
20:0	15.4	-	-	-	-	-	-	-	-	-	0.70	0.45
22:0	12.2	0.51	0.39	0.66	0.21	0.31	0.26	0.40	0.25	0.32	0.46	0.42
24:0	16.1	0.61	0.41	0.78	0.27	0.36	0.28	0.41	0.34	0.59	0.41	0.60
26:0	20.6	0.57	0.43	1.12	0.39	0.70	0.49	0.43	0.50	1.04	0.59	0.62
28:0	-	-	-	-	-	0.47	0.38	-	-	-	-	-
Δ9-Desaturates												
18:1	10.4	9.19	4.24	7.48±0.15	Tr.	3.84	7.08±0.23	4.16±0.11	5.25±1.03	5.53±0.83	3.77±0.42	5.13±0.31
ω6-Fatty acids												
18:2	10.2	12.18±1.03	3.47	4.10	5.62	1.91	Tr.	3.97±0.73	2.79±0.57	3.20±0.38	Tr.	-
Yield, %		2.73	1.86	1.68	11.95	2.18	1.94	5.11	2.57	1.69	1.32	2.33
$\Sigma_{Sat.}$		14.98	5.02	7.91	6.28	4.59	3.02	5.03	3.15	4.70	8.47	5.07
$\Sigma_{Unsat.}$		21.37	7.71	11.58	5.62	5.75	7.08	8.13	8.04	8.73	3.77	5.13
U/S		1.43	1.54	1.46	0.89	1.25	2.34	1.62	2.55	1.86	0.45	0.06
Total		36.15	12.73	19.49	11.90	10.34	10.10	13.16	11.19	13.43	12.24	10.20

*Means with the same letter in each row do not significantly differ at 0.06 level.

Host plants: AVA: *Armeniaca vulgaris* (Apricot), AVW: *Armeniaca vulgaris* (Wild Apricot), PE: *Pyrus eleagnifolia* ssp. *eleagnifolia* (Wild pear), PC: *Pyrus communis* ssp. *communis* ssp. *sativa* (Pear), RP: *Robinia pseudoacacia* (Acacia), CO: *Cydonia oblonga* (Quince), CA: *Cerasus avium* (Cherry), CV: *Cerasus vulgaris* (Sour cherry), PD: *Prunus domestica* (Plum), PN: *Pinus nigra* (Pine), AB: *Abies bornmulleriana* (Fir).

According to the results obtained, a larger variation for the amounts of palmitic (C16:0), oleic (C18:1), and linoleic (C18:2) acids was observed in this study. GC-MS analysis demonstrated that, among the saturated fatty acids, *V. album* subspecies generally contain palmitic (1.20–11.47%), stearic (0.70–1.62%), cerotic (0.39–1.12%), lignoseric (0.27–0.78%), and behenic acids (0.21–0.66%). The sample of *V. album* ssp. *album* growing on apricot tree was remarkably rich in palmitic acid (11.47%) followed by ssp. *austriacum* sample (5.29%) on pine tree and ssp. *album* sample (4.49%) on pear tree. But, palmitic acid was not detected in ssp. *album* sample growing on quince tree. On the other hand, arachidic acid was found in ssp. *austriacum* and ssp. *abietis* samples, whereas montanic acid was found in only ssp. *album* samples growing on quince and acacia trees. The dominant fatty acid in all the samples was oleic acid. Its content ranged between 3.77 and 9.19%. The lipophylic extracts of *V. album* ssp. *album* samples were rich in terms of unsaturated fatty acids (especially oleic and linoleic acids) (Table 1). *V. album* ssp. *austriacum* and ssp. *abietis* samples also contained oleic acid, while linoleic acid was only detected in *V. album* ssp. *album* subspecies. The highest amount of unsaturated fatty acids (9.19% for oleic acid and 12.18% for linoleic acid) was found to be in ssp. *album* sample growing on apricot trees. The ratio of unsaturated fatty acids to the saturated (U/S ratio) ranged between 0.06 to 2.55.

The proximate analysis of fatty acid compositions (mean and standard error values) of the lipophylic extracts by GC-MS is presented in Table 1.

Studies by other researchers have demonstrated that apricot, plum, cherry, and sour cherry contain high amounts of linoleic (34.5–70.5%) and linolenic acids (15.7–45.7%) [7, 8].

But, in this study, linolenic acid was not detected in the samples collected from these host trees. On the other hand, Hafizoglu et al. studied the lipophylic constituents of barks from *Pinus nigra* and *Abies bornmulleriana* and found that *A. bornmulleriana* possessed 34.5% fatty acids in total, which consisted of palmitic, margaric, stearic, lignoseric, oleic, eicosatrienoic, and linoleic acids, while *P. nigra* contained 23.2% fatty acid in total, consisting of palmitic, margaric, linoleic, oleic, arachidic, behenic, eicosatrienoic, and lignoseric acids [9]. However, the present study showed that ssp. *abietis* (on *A. bornmulleriana*) and ssp. *austriacum* samples (on *P. nigra*) contained oleic, palmitic, stearic, arachidic, behenic, lignoseric, and cerotic acids. But, margaric and eicosatrienoic acids were not detected in these samples.

Some vital functions of EFAs are the production of prostaglandins, which regulate body functions such as heart rate, blood pressure, blood clotting, fertility, and conception and play a role in immune function by regulating inflammation and encouraging the body to fight infection [10]. Ergun et al. studied the vascular effects of petroleum ether extracts of the same *V. album* samples on an isolated rat aortic ring preparation and found that the petroleum ether extract obtained from ssp. *album* sample on growing apricot tree produced marked vasodilator activity when compared with sodium nitroprusside (SNP) [11]. Interestingly, in this study, the highest amount of linoleic acid was found to be in ssp. *album* sample growing on apricot trees.

In the previous studies on the fatty acid composition of *V. album*, the presence of oleic, palmitic, and linoleic acids has been reported in the plant [2, 3]. But the subspecies of *V. album* were not taken into consideration. The subspecies of *V. album* samples were appraised in our study in order to investigate the fatty acid composition of the plant.

According to the results, no remarkable correlation among the fatty acid compositions of three *V. album* subspecies was observed with respect to their host plants. In other words, we postulate that the composition of the lipophylic extracts of *V. album* samples is not dependent on their host trees.

EXPERIMENTAL

Plant Materials. The host plants, localities, and collection time of *V. album* L. subspecies were as follows: ssp. *album* from *Armeniaca vulgaris* Lam. (Wild apricot), *Cydonia oblonga* Miller (Quince), *Cerasus avium* L. (Moench.) (Cherry), *Cerasus vulgaris* Miller (Sour cherry), Iskilip, Corum, June 1995, from *Armeniaca vulgaris* Lam. (Apricot), *Pyrus communis* L. ssp. *communis* ssp. *sativa* (DC.) Hegi (Pear), *Robinia pseudoacacia* L. (Acacia), Baglum, Ankara, June 1995, from *Pyrus eleagnifolia* Pallas ssp. *eleagnifolia* (Wild pear), Kizilcahamam road, Ankara, April 1995, from *Prunus domestica* L. (Plum), Aglasun, Isparta, April 1995; ssp. austriacum from *Pinus nigra* Arn., (Pine), Kizilcahamam, Ankara, June 1995; ssp. *abietis* from *Abies bornmulleriana* Mattf. (Fir), Golcuk Lake, Bolu, June 1995. The plants were identified by Prof. Dr. Mecit Vural of the Department of Botany, Faculty of Science and Art, Gazi University, Ankara, Turkey, and voucher specimens of the plants are kept in the herbarium of the Faculty of Pharmacy, Ankara University, Ankara, Turkey (AEF).

Preparation of the Lipophylic Extracts. Each plant part (leaves, twigs, and stems) used in this study was dried under shade, ground in a mechanic grinder to a fine powder, and weighed accurately (5 g for each). The powdered plant parts were macerated with 100 mL of *n*-hexane (Merck Co., USA) at room temperature for 2 days and shaken occasionally by hand.

Then, the *n*-hexane phase of each extract was filtered through a filter paper and evaporated in reduced pressure until dryness (Büchi, Switzerland). The obtained lipophylic extracts were weighed accurately again in an analytical scale (Shimadzu, Libror AEG-120) and percentage yields (w/w) were calculated.

Saponification and Derivatization. The lipophylic extracts were independently saponified with 0.5 N methanolic NaOH solution by heating on a steam bath until fat globules disappeared on the surface of the solution, an approximately five-minute step, then boiled for 2 minutes. Then, 20 mL of boron trifluoride-methanol complex reagent (20%, Merck Co.) was subsequently added and the solutions kept for another 2 minutes again in a boiling water bath. After cooling, each solution was completed saturated with NaCl solution in 25 mL measuring flasks. The mixtures were left for 30 minutes for gathering the oily part on the surface of the solution and converted to their methyl ester forms prior to GC-MS analysis [12].

Conditions of GC-MS Analysis. Chromatographic analysis was carried out on a Hewlett Packard HP 6890 series GC-MS apparatus combined with a mass selective detector. The capillary column used was an HP-5MS (5% phenyl methylsiloxane, 30.1 m $\times$ 250 μm $\times$ 0.25 μm). Helium was used as carrier gas at a flow rate of 1.0 mL/min with 5 mL injection volume. Samples were analyzed with the column held initially at 180°C for 0.5 min after injection, then increased to 240°C with 8°C/min heating ramp with 1 min hold time. Then final the temperature was increased to 300°C with 2°C/min heating ramp for 10 min. The injection was performed in split mode (split ratio: 10:1). Detector and injector temperatures were 250°C and 280°C, respectively.

Run time was 49 min. MS scan range was (*m/z*): 20–440 atomic mass units (AMU) under electron impact (EI) ionization (70 eV). Ion source temperature: 250°C. All injections were done in triplicate.

Identification of the Fatty Acids. The fatty acid components of the lipophylic extracts were determined by comparing their mass fragmentations with those of mass spectra from the Wiley database search as well as comparison of the retention times and mass spectrums of authentic samples of the fatty acids.

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