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Short Communication

Fatty Acid Profile of Roots and Aerial Parts of *Ruscus hyrcanus* Woronow

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ABSTRACT

Background: *Ruscus* specie are used as traditional medicine, food, and foliage. The aim of this work is the determination of fatty acid composition of *Ruscus hyrcanus* as a native medicinal plant of Iran for the first time together with comparison of different esterification methods.

Methods: Two different esterification methods were used for preparation of esterified fatty acids from different extracts of underground and aerial parts of the herb. GC/MS analysis were used for identification and quantification of fatty acids. Finally, the results were compared.

Results: Findings showed that *R. hyrcanus* is rich in essential fatty acids such as linoleic acid (13-25%) and linolenic acid (23-44%). Also, oil samples contain remarkable amount of palmitic acid (19-57%).

Conclusion: The results showed that *R. hyrcanus* could be considered as a source of essential fatty acids. Also, it could be concluded that a simple esterification method with methanolic KOH and 2 min vortex is suitable for fatty acid analysis of *Ruscus* species.

Introduction

The genus *Ruscus* belongs to Asparagaceae family and is native to Europe and western Asia. This genus contains 7 species distributed from Europe to Iran and is represented by perennial, rhizomatous, and evergreen shrubs.[1] The aerial parts of these plants are edible but the underground parts (rhizome and roots) are used in traditional medicine of many countries for treatment of several illnesses such as inflammation, hemorrhoids, atherosclerosis, chronic venous insufficiency, vasculitis, nephritis, eczema, warts, chilblains, arthritis, colitis, diarrhea, and skin disorders.[2-10] *Ruscus hyrcanus* is used as diuretic, appetizer, antibleeding, vasoconstrictor, anti-infection, antinephritis, antivaricose, aperient, and laxative agent in Iranian folk medicine.[11]

Besides studies reporting the traditional uses of *Ruscus* species, other *in vitro* and *in vivo* studies indicated the pharmacological activities of these plants such as antimicrobial, antifungal, antioxidant, lactogenic, and anti-inflammatory effects.[5,9,12,13] Todays, a number of products are developed based on *R. aculeatus* extracts which are acting on the venous system.[14-17] These products have a great market in Europe and are utilized for management of vascular diseases.[18-20]

There are many phytochemical analyses on *Ruscus* species indicating the presence of several classes of phytochemicals in these medicinal plants. The main secondary metabolites in the genus *Ruscus* are steroidal saponins such as spirostanol, furostanol and cholestane saponins.[21] These natural products are considered to be active agents of extracts and commercial products of *Ruscus* plants in the market. Moreover, two spirostane aglycones ruscogenin and neoruscogenin are known as responsible compounds for many biological properties of *Ruscus* species.[22,23]

Lipids have a critical role in human diet because they provide energy and essential fatty acids.[24,25] Furthermore, they are important for carrying lipid soluble vitamins, and synthesis of prostaglandins and steroidal hormones.[26] Quality and quantity of fatty acids play vital role in human health.[27] They differ in chain length, position, and number of double bonds as well as *cis*/*trans* orientation. Fatty acids are classified as short chain (2–8), middle chain (8–12), and long chain (13–24). Also, fatty acids could be classified based on saturation degree as saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids.[28] They are associated with cardiovascular diseases due to their major role in cholesterol synthesis.[29-31] Linoleic acid and α-linolenic acid are called "essential fatty acids" because human body could not produce them and they should be taken from diet.[31] Fatty acids could be found in little amounts in free form but generally they are composed in some complex structures such as cholesterol esters, acylglycerols, waxes, and glycosphingolipids through amide or ester bonds.[32,33]

Generally, the fatty acid (FA) composition of biological materials is determined using gas chromatography-mass spectrometry (GC-MS) as fatty acid methyl esters (FAME) . For the analysis of FAs by GC-MS it is necessary to increase their volatility using derivatisation

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methods. This could be obtained through transesterification by using of an alkyl ester.[34] FAME could be prepared by different methods including acid-catalyzed and alkali-catalyzed methanolysis, and methylation after saponification of glycerolipids.[35] Among the various procedures for preparation of FAME, KOH-catalyzed methanolysis is a fast and simple method. Other conventional methods of alkali/acid-catalyzed methanolysis in which samples are refluxed are time-consuming methods.[36]

There is a lack of information about chemical and biological properties of *Ruscus* species because most of investigations focused on *R. aculeatus*. Besides these studies, a few other reports on the pharmacological activity and chemical composition of *Ruscus* species have been performed so far. Accordingly, this work aimed to evaluate the fatty acid profile of *Ruscus hyrcanus* for the first time. To the best of our knowledge, this is also the first report on fatty acid composition of the genus *Ruscus*.

Materials and Methods

Plant material

The plant materials were collected from Golestan province (North of Iran) and taxonomically identified by Dr. Hossein Nazemiyeh in the Herbarium of Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran. A voucher specimen was also deposited (TbZ FPh-399).

Extraction of fatty acids

The extraction of FAs from 5 g dried and powdered plant samples (aerial parts and underground parts of *Ruscus hyrcanus*) was successively performed at boiling point for 8 h using a Soxhlet apparatus by petroleum ether and chloroform as the solvents. Subsequently, extracts were filtered through a filter paper and the solvent was removed using rotary evaporator at 40 °C under reduced pressure. Samples stored in dark at 4 °C until analysis.

Preparation of FAME (Esterification)

Preparation of fatty acid methyl ester derivatives was carried out using two different methods. In the first procedure, 10 mg of extracts were placed in small glass tubes and 2 mL *n*-hexane and 0.2 mL methanolic KOH (2 M) were added to the tubes. Afterward, the tube was vortexed for 2 min at room temperature for methanolysis reaction. An aliquot of the upper layer (*n*-hexane) of mixture in the reaction tube was directly injected to GC-MS. The second method was similar to the first with one difference. In the second procedure, 30 min reflux was used for completing the reaction instead of vortex step.

Determination of fatty acid composition

Fatty acid composition was investigated by gas chromatography-mass spectrometry technique (Shimadzu, QP-5050 A) using DB-1 capillary column (60 m, 0.25 mm i.d., 0.25 µm). Electron impact ionization system (ionization energy = 70 eV) was applied for identification of volatile derivatives. Analysis condition was as follow: Carrier gas: Helium, Flow rate: 1 mL min^{-1}, Linear velocity = 29.6 cm/s, Split ratio = 1:20. Temperature program of column: the initial oven temperature = 50 °C for 3 min, then raising from 50 °C to 265 °C with program ramp rate of 2.5 °C/min. The final temperature was 265 °C and was kept for 6 °C. The injector temperature was 250 °C. Assessment of FAs was performed by comparison of relative mass spectra from sample FAME peaks with those of Wiley 229, Nist 107, Nist 21, and Adams 2007 Libraries. Results were expressed as Mass response area in relative percentages.

Results and Discussion

The oil extraction using petroleum ether and chloroform yielded 2.1% and 6.2% (w/w) for the aerial parts, and 2.5% and 8.5% for the roots of the plant, respectively. A total of 11 fatty acids were detected in the extracts of aerial parts and roots of *Ruscus hyrcanus* which ranged from C 12:0 to C 24:0. The results are shown in Table 1. GC/MS analysis revealed that the main fatty acids of *Ruscus hyrcanus* were 9,12-Octadecadienoic acid (Linoleic acid) (23% to 44%), Hexadecanoic acid (Palmitic acid) (19% to 57%), and 9-Octadecenoic acid (Oleic acid) (10% to 25%). Linoleic acid and palmitic acid exist in all samples. Generally, the number of identified FAs in *n*-hexane samples is more than chloroform samples. Also, the number of FAs in root samples is more than those of aerial parts.

Table 1. Fatty acid composition of *n*-hexane and chloroform extracts of *R. hyrcanus*.

No.	Fatty acid			Concentration (%)							
				Roots				Aerial parts			
				Hexane		Chloroform		Hexane		Chloroform	
				V[a]	R[b]	V	R	V	R	V	R
1	Dodecanoic acid (Lauric acid)	C 12:0		0.78	-	-	-	1.13	-	-	-
2	Tetradecanoic acid (Myristic acid)	C 14:0		1.1	-	-	-	1.64	-	-	-
3	Hexadecanoic acid (Palmitic acid)	C 16:0		19.17	20.66	39.72	41.02	24.19	24.35	55.91	57.26
4	Octadecanoic acid (Stearic acid)	C 18:0		2.07	3.17	3.7	4.1	4.74	5.71	-	-
5	Eicosanoic acid (Arachidic acid)	C 20:0		0.92	-	-	-	2.27	-	-	-
6	Docosanoic acid (Behenic acid)	C 22:0		0.86	-	-	-	2.98	5.8	-	-
7	Tetracosanoic acid (Lignoceric acid)	C 24:0		0.57	-	-	-	-	-	-	-
8	9,12-Hexadecadienoic acid	C 16:2	ω4	22.61	-	-	-	-	-	-	-
9	9-Octadecenoic acid (Oleic acid)	C 18:1	ω9	13.72	15.33	10.7	10.5	22.07	25.52	-	-
10	9,12-Octadecadienoic acid (Linoleic acid)	C 18:2	ω6	23.75	42.59	39.4	37.45	36.92	33.07	44.09	42.74
11	9,12,15-Octadecatrienoic acid (Linolenic acid)	C 18:3	ω3	2.13	-	-	-	3.08	-	-	-

a: Vortexed (esterification method 1), b: Refluxed (esterification method 2).

Table 2. Fatty acid types in different assays.

No.	Concentration (%)							
	Roots				Aerial parts			
	Hexane		Chloroform		Hexane		Chloroform	
	V	R	V	R	V	R	V	R
ΣSFA[c]	25.47	23.83	43.42	45.12	36.95	35.86	55.91	57.26
ΣMUFA[d]	13.72	15.33	10.7	10.5	22.07	25.52	-	-
ΣPUFA[e]	48.49	42.59	39.47	37.45	40.0	33.07	44.09	42.74
ΣUFA[f]	62.21	57.92	50.17	47.95	62.07	58.59	44.09	42.74
ΣEFA[g]	25.88	42.59	39.47	37.45	40.0	33.07	44.09	42.74
Σω3	2.13	-	-	-	3.08	-	-	-
Σω6	23.75	42.59	39.47	37.45	36.92	33.07	44.09	42.74
Σω9	13.72	15.33	10.7	10.5	22.07	25.52	-	-
ω3/ω6	0.09	-	-	-	0.083	-	-	-
ω6/ω3	11.15	-	-	-	11.99	-	-	-
ω6/ω9	1.73	2.78	3.69	3.57	1.67	1.29	-	-
Oil yield (%)	2.4		8.5		2.1		6.2	
Total identified	87.68	81.75	93.59	93.07	99.02	94.45	100	100

a: Vortexed (esterification method 1), b: Refluxed (esterification method 2).
c: saturated fatty acids, d: monounsaturated fatty acids, e: polyunsaturated fatty acids, f: unsaturated fatty acids, g: essential fatty acids

All samples were rich in unsaturated fatty acids (UFA) (47% to 62%) except of chloroform samples of aerial parts which were richer than *n*-hexane samples in saturated fatty acids (SFA) (55% to 57%). In addition, the percentage of poly-unsaturated fatty acids (PUFAs) was more than mono-unsaturated fatty acids (MUFAs) in all oil samples. Just 1 MUFA (Oleic acid) was detected in the samples. Linoleic acid was the dominant PUFA (23% to 44%). All tested extracts exhibited high level of essential FAs (linoleic acid and linolenic acid) ranging from 25 to 44%. Furthermore, the ratios of omega3/omega6, omega6/omega3, and omega6/omega9 were also calculated (Table 2).

According to the results, the first method (2 min vortex) could be suitable for FA analysis of *Ruscus* species. The second method (30 min reflux) has not any advantage to the first one and also is time and energy consuming. Moreover, generally, the number of detectable FAs in assays using the first method is more than those of second method (Table 1).

Conclusion

There is not any published data about FA composition of *Ruscus* species. So, the present work is the first report in scientific area. This could be important due to wide consumption and the nutritional and medicinal value of the genus. GC/MS analysis revealed that *R. hyrcanus* is rich in essential fatty acids and has a balanced ratio of saturated and unsaturated FAs. This study also showed the variation for total content of FAs among the *R. hyrcanus* samples obtained by different methods. In addition, this species has a high content of linoleic and linolenic acid. So, this herb may also be considered as a new source of essential FAs. Further studies should be performed to determine the phytochemical and pharmacological properties of this species.

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Conflict of interests

The authors claim that there is no conflict of interest.

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