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

Mir Babak Bahadori1, Solmaz Asnaashari2, Hossein Nazemiyeh3,4

1Medicinal Plants Research Center, Maragheh University of Medical Sciences, Maragheh, Iran.
2Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
3Research Center for Pharmaceutical Nanotechnology and Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.

**Abstract**

*Background:* *Ruscus* species 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.
Fatty acid Composition of Ruscus hyrcanus

Fatty acid methyl ester derivatives were prepared using two different methods. In the first procedure, 10 mg of extracts were placed in small glass tubes and 2 mL of 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 those of chloroform samples. Also, the number of FAs in root samples is more than those of aerial parts.

| No. | Fatty acid                  | Concentration (%) |
|-----|-----------------------------|-------------------|
|     | Hexane | Chloroform | Hexane | Chloroform |
|-----|---------|------------|--------|------------|
| 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 | 22.61 | - | - | - | - | - | - |
| 9   | 9-Octadecenoic acid (Oleic acid) | C 18:1 | 13.72 | 15.33 | 10.7 | 10.5 | 22.07 | 25.52 | - | - |
| 10  | 9,12-Octadecadienoic acid | C 18:2 | 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 | 2.13 | - | - | 3.08 | - | - | - |

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

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

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 of n-hexane and 0.2 mL methanolic KOH (2% w/v) 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.

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 those of chloroform samples. Also, the number of FAs in root samples is more than those of aerial parts.
All samples were rich in unsaturated fatty acids (UFA) (47% to 62%) except of chlorofrom 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 (MUFA) 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/omega9, and omega6/omega3 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.

### Acknowledgements

The financial assistance from Tabriz University of Medical Sciences is gratefully acknowledged. Also, the authors would like to acknowledge the support of Ministry of Health and Medical Education.

### Table 2. Fatty acid types in different assays.

| No. | Roots | Aerial parts |
|-----|-------|-------------|
|     | Hexane | Chloroform  | Hexane | Chloroform  |
|     | V     | R           | V     | R           |
| $\Sigma$SFA$^a$ | 25.47 | 23.83 | 43.42 | 45.12 | 36.95 | 35.86 |
| $\Sigma$MUFA$^b$ | 13.72 | 15.33 | 10.7 | 10.5 | 22.07 | 25.52 |
| $\Sigma$PUFA$^c$ | 48.49 | 42.59 | 39.47 | 37.45 | 40.0 | 33.07 |
| $\Sigma$EFA$^d$ | 62.21 | 57.92 | 50.17 | 47.95 | 62.07 | 58.59 |
| $\Sigma$SFA | 25.88 | 42.59 | 39.47 | 37.45 | 40.0 | 33.07 |
| $\Sigma$FA | 2.13 | - | - | - | 3.08 | - |
| $\Sigma$SF | 23.75 | 42.59 | 39.47 | 37.45 | 36.92 | 33.07 |
| $\Sigma$MUFA | 13.72 | 15.33 | 10.7 | 10.5 | 22.07 | 25.52 |
| $\Sigma$PUFA | 0.09 | - | - | - | 0.083 | - |
| $\Sigma$EFA | 11.15 | - | - | - | 11.99 | - |
| $\Sigma$FA | 1.73 | 2.78 | 3.69 | 3.57 | 1.67 | 1.29 |
| Oil yield (%) | 2.4 | - | 8.5 | - | 2.1 | - |
| Total identified | 87.68 | 81.75 | 93.59 | 93.07 | 99.02 | 94.45 |

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

### Conflict of interests

The authors claim that there is no conflict of interest.

### References

1. Thomas PA, Mukassabi TA. Biological flora of the british isles: Ruscus aculeatus. J Ecol. 2014;102(4):1083-100. doi:10.1111/1365-2745.12265
2. Ali-Shtayeh MS, Yaghmour RM, Faidi YR, Salem K, Al-Nuri MA. Antimicrobial activity of 20 plants used in folkloric medicine in the palestinian area. J Ethnopharmacol. 1998;60(3):265-71. doi:10.1016/S0378-8741(97)00153-0
3. Bouskela E, Cyrino FZ, Marcelon G. Possible mechanisms for the inhibitory effect of ruscus extract on increased microvascular permeability induced by histamine in hamster cheek pouch. J Cardiovasc Pharmacol. 1994;24(2):281-5. doi:10.1097/0001378-8741(97)00153-0
4. Guarrera PM. Traditional phytotherapy in central italy (marche, abruzzo, and latium). Fitoterapia. 2005;76(1):1-25. doi:10.1016/j.fito.2004.09.006
5. Hadžifejzović N, Kukić-Marković J, Petrović S, Soković M, Glamočlija J, Stojković D, et al. Bioactivity of the extracts and compounds of ruscus aculeatus L. and ruscus hypoglossum L. Ind Crops Prod. 2013;49:407-11. doi:10.1016/j.indcrop.2013.0.5.036
6. Huang Y-L, Kou J-P, Lu L, Song J-X, Yu B-Y. Possible mechanism of the anti-inflammatory activity of ruscogenin: Role of intercellular adhesion molecule-1 and nuclear factor-kb. J Pharmcol Sci. 2008;108(2):198-205. doi:10.1254/jphs.08083FP
7. Longo L, Vasapollo G. Determination of anthocyanins in ruscus aculeatus L. Berries. J Agric Food Chem. 2005;53(2):475-9. doi:10.1021/jf0487250
8. Bouskela E, Cyrino FZ, Marcelon G. Inhibitory effect of the ruscus extract and of the flavonoid hesperidine methylchalcone on increased microvascular permeability induced by various agents in the hamster cheek pouch. J Cardiovasc Pharmacol. 1993;22(2):25-30. doi:10.1097/00005344-199308000-00009
9. Balica G, Vostinaru O, Tamas M, Crisan G, Mogosan
C. Anti-inflammatory effect of the crude steroidal saponin from the rhizomes of ruscus aculeatus l.(ruscaceae) in two rat models of acute inflammation. J Food Agric Environ. 2013;11(3-4):106-8.

10. Güvenç A, Şaatir E, Coşkun M. Determination of ruscogenin in turkish ruscus l. Species by uplc. Chromatographia. 2007;66(S1):141-5. doi: 10.1365/s10337-007-0351-2

11. Dehghan H, Sarrafi Y, Salehi P. Antioxidant and anti diabetic activities of 11 herbal plants from hycania region, iran. J Food Drug Anal. 2016;24(1):179-88. doi: 10.1016/j.jfda.2015.06.010

12. Maswadeh HM, Semreen MH, Naddaf AR. Anti-inflammatory activity of achillea and ruscus topical gel on carrageenan-induced paw edema in rats. Acta Pol Pharm. 2006;63(4):277-80.

13. Kemertelidze EP, Muzashvili TS, Benidze MM, Tsaruk AV, Khushbaktova ZA, Syrov VN. Chemical composition and pharmacological activity of ruscus colchicus leaves. Pharm Chem J. 2012;46(6):372-5. doi: 10.1007/s11094-012-0801-5

14. Marcelon G, Verbeuren TJ, Laressergues H, Vanhoutte PM. Effect of ruscus aculeatus on isolated canine cutaneous veins. Gen Pharmac. 1983;14(1):103-6. doi: 10.1016/0306-3623(83)900745

15. Rudofsky G. Improving venous tone and capillary sealing. Effect of a combination of ruscus extract and hesperidin methyl chalcone in healthy probands in heat stress. Fortschr Med. 1989;107(1959):52:55-8.

16. Marcelon G, Pouget G, Tisverserailles J. Alpha-adrenergic responsiveness on canine thoracic-duct lymph-effect of ruscus aculeatus extract. Blood Vessels. Switzerland: Karger Allschwilerstrasse 10, CH-4009 Basel; 1987.

17. Facino RM, Carini M, Stefani R, Aldini G, Saibene L. Anti-elastase and anti-hyaluronidase activities of saponins and sapogenins from hedera helix, aesculus hippocastanum, and ruscus aculeatus: Factors contributing to their efficacy in the treatment of venous insufficiency. Arch Pharm. 1995;328(10):720-4. doi: 10.1002/ardp.19953281006

18. Boisseau MR. Pharmacological targets of drugs employed in chronic venous and lymphatic insufficiency. Int Angiol. 2002;21(2 Suppl 1):33-9.

19. Svensjö E, Bouskela E, Cyrino FZ, Bougaret S. Antipermeability effects of cyclo 3 fort in hamsters with moderate diabetes. Clin Hemorheol Microcirc. 1997;17(5):385-8.

20. Janssens D, Delaive E, Houbion A, Eliaers F, Remacle J, Michiels C. Effect of venotropics drugs on the respiratory activity of isolated mitochondria and in endothelial cells. Br J Pharmacol. 2000;130(7):1513-24. doi: 10.1038/sj.bjp.0703461

21. Masullo M, Pizza C, Piccante S. Ruscus genus: A rich source of bioactive steroidal saponins. Planta Med. 2016;82(18):1513-24. doi: 10.1055/s-0042-119728

22. de Combarieu E, Falzoni M, Fuzatti N, Gattesco F, Giori A, Lovati M, et al. Identification of ruscus steroidal saponins by hplc-ms analysis. Fitoterapia 2002;73(7-8):583-96. doi: 10.1016/S0367-326X(02)00220-4

23. Boyle P, Diehm C, Robertson C. Meta-analysis of clinical trials of cyclo 3 fort in the treatment of chronic venous insufficiency. Int Angiol. 2003;22(3):250-62.

24. Li X-Q, Song A-H, Li W, Chen X-H, Bi K-S. Analysis of the fatty acid from bupleurum chinense dc in china by gc-ms and gc-fid. Chem Pharm Bull. 2005;53(12):1613-7. doi: 10.1248/cpb.53.1613

25. Aktumsek A, Zengin G, Guler GO, Cakmak YS, Duran A. Screening for in vitro antioxidant properties and fatty acid profiles of five centaurea l. Species from turkey flora. Food Chem Toxicol. 2011;49(11):2914-20. doi: 10.1016/j.fct.2011.08.016

26. Li X, Kong W, Shi W, Shen Q. A combination of chemometrics methods and gc-ms for the classification of edible vegetable oils. Chemometr Intell Lab Syst. 2016;155:145-50. doi: 10.1016/j.chemolab.2016.03.028

27. Dorni C, Sharma P, Saikia G, Longvah T. Fatty acid profile of edible oils and fats consumed in india. Food Chem. 2018;238:9-15. doi: 10.1016/j.foodchem.2017.05.072

28. Zengin G, Cakmak YS, Guler GO, Aktumsek A. In vitro antioxidant capacities and fatty acid compositions of three centaurea species collected from central anatolia region of turkey. Food Chem Toxicol. 2010;48(10):2638-41. doi: 10.1016/j.fct.2010.06.033

29. Connor WE. Importance of n–3 fatty acids in health and disease. Am J Clin Nutr. 2000;71(1):171S-5S. doi: 10.1093/ajcn/71.1.171S

30. Mozaffarian D, Kutan MB, Ascherio A, Stampfer MJ, Willett WC. Trans fatty acids and cardiovascular disease. New Engl J Med. 2006;354(15):1601-13. doi: 10.1056/NEJMra054035

31. Simopoulos AP. Essential fatty acids in health and chronic disease. Am J Clin Nutr. 1999;70(3):560s-9s. doi: 10.1093/ajcn/70.3.560s

32. Larsson SC, Kunlin M, Ingelman-Sundberg M, Wolk A. Dietary long-chain n–3 fatty acids for the prevention of cancer: A review of potential mechanisms. Am J Clin Nutr. 2004;79(6):935-45. doi: 10.1093/ajcn/79.6.935

33. Lowry RR, Tinsley JJ. Rapid colorimetric determination of free fatty acids. J Am Oil Chem Soc. 1976;53(7):470-2. doi: 10.1007/BF02636814

34. Ichihara K, Shibahara A, Yamamoto K, Nakayama T. An improved method for rapid analysis of the fatty acids of glycerolipids. Lipids. 1996;31(5):535-9. doi: 10.1007/BF02522648

35. Christie WW. The Preparation of Alkyl Esters from Fatty Acids and Lipids. In: Gunstone FD, Elek P, editor. Topics in Lipid Chemistry, Vol. 3. London: Scientific Books Ltd;1972. p. 171-197

36. Liu K-S. Preparation of fatty acid methyl esters for gas-chromatographic analysis of lipids in biological materials. J Am Oil Chem Soc. 1994;71(11):1179-87. doi: 10.1007/BF02540534