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

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Introduction

The genus Ruscus belongs to Asparagaceae family and is native to Europe and native 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, antiviscaricose, 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 Today, 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 cholestanol saponins.21 These natural products are considered to be active agents of extracts and commercial products of Ruscus plants in the market. Moreover, two spirostanol 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 steroid hormonal.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
methods. This could be obtained through transesterification by using of an alkyl ester. FAME could be prepared by different methods including acid-catalyzed and alkali-catalyzed methanolysis, and methylation after saponification of glycerolipids. 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.

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 methanolyis 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-Octodecadienoic acid (Linoleic acid) (23% to 44%), Hexadecanoic acid (Palmitic acid) (19% to 57%), and 9-Octadeconoic 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.

<table>
<thead>
<tr>
<th>No.</th>
<th>Fatty acid</th>
<th>Concentration (%)</th>
<th>Hexane</th>
<th>Chloroform</th>
<th>Hexane</th>
<th>Chloroform</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dodecanoic acid (Lauric acid)</td>
<td>C 12:0</td>
<td>0.78</td>
<td>-</td>
<td>1.13</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Tetradecanoic acid (Myristic acid)</td>
<td>C 14:0</td>
<td>1.1</td>
<td>-</td>
<td>1.64</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Hexadecanoic acid (Palmitic acid)</td>
<td>C 16:0</td>
<td>19.17</td>
<td>20.66</td>
<td>39.72</td>
<td>41.02</td>
</tr>
<tr>
<td>4</td>
<td>Octadecanoic acid (Stearic acid)</td>
<td>C 18:0</td>
<td>2.07</td>
<td>3.17</td>
<td>3.7</td>
<td>4.1</td>
</tr>
<tr>
<td>5</td>
<td>Eicosanoic acid (Arachidic acid)</td>
<td>C 20:0</td>
<td>0.92</td>
<td>-</td>
<td>2.27</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Docosanoic acid (Behenic acid)</td>
<td>C 22:0</td>
<td>0.86</td>
<td>-</td>
<td>2.98</td>
<td>5.8</td>
</tr>
<tr>
<td>7</td>
<td>Tetracosanoic acid (Lignoceric acid)</td>
<td>C 24:0</td>
<td>0.57</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>9,12-Hexadecadienoic acid</td>
<td>C 16:2 ω4</td>
<td>22.61</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>9-Octodecenoic acid (Oleic acid)</td>
<td>C 18:1 ω9</td>
<td>13.72</td>
<td>15.33</td>
<td>10.7</td>
<td>10.5</td>
</tr>
<tr>
<td>10</td>
<td>9,12-Octadecadienoic acid (Linoleic acid)</td>
<td>C 18:2 ω6</td>
<td>23.75</td>
<td>42.59</td>
<td>39.4</td>
<td>37.45</td>
</tr>
<tr>
<td>11</td>
<td>9,12,15-Octadecestrienoic acid (Linolenic acid)</td>
<td>C 18:3 ω3</td>
<td>2.13</td>
<td>-</td>
<td>-</td>
<td>3.08</td>
</tr>
</tbody>
</table>

a: Vortexed (esterification method 1), b: Refluxed (esterification method 2).
The number of detectable FAs in her studies should be performed to J Ecol.

Ministry of Health and Medical Education authors would like to

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properties of this species.

determine the phytochemical and pharmacological

So, this herb may also be considered as a new source of

species has a high content of linoleic and linolenic acid.

samples obtained by different methods. In addition, this

variation for total content of FAs among the

saturated and unsaturated FAs. This study also sho

rich in essential fatty acids and has a balanced ratio of

consumption and the nutritional and medicinal value of

scientific area

Conclusion

According to the results, the first method (2 min vortex)

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).

Table 2. Fatty acid types in different assays.

<table>
<thead>
<tr>
<th>No.</th>
<th>Roots</th>
<th>Chloroform</th>
<th>Aerial parts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hexane</td>
<td>V</td>
<td>R</td>
</tr>
<tr>
<td>ΣSFAa</td>
<td>25.47</td>
<td>23.83</td>
<td>43.42</td>
</tr>
<tr>
<td>ΣMUFAa</td>
<td>13.72</td>
<td>15.33</td>
<td>10.7</td>
</tr>
<tr>
<td>ΣPUFAa</td>
<td>48.49</td>
<td>42.59</td>
<td>39.47</td>
</tr>
<tr>
<td>ΣFA</td>
<td>62.21</td>
<td>57.92</td>
<td>50.17</td>
</tr>
<tr>
<td>ΣEFAa</td>
<td>25.88</td>
<td>42.59</td>
<td>39.47</td>
</tr>
<tr>
<td>Σω3</td>
<td>2.13</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Σω6</td>
<td>23.75</td>
<td>42.59</td>
<td>39.47</td>
</tr>
<tr>
<td>Σω9</td>
<td>13.72</td>
<td>15.33</td>
<td>10.7</td>
</tr>
<tr>
<td>ω3ω6</td>
<td>0.09</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ω6ω3</td>
<td>11.15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ω6ω9</td>
<td>1.73</td>
<td>2.78</td>
<td>3.69</td>
</tr>
<tr>
<td>Oil yield (%)</td>
<td>2.4</td>
<td>8.5</td>
<td>2.1</td>
</tr>
<tr>
<td>Total identified</td>
<td>87.68</td>
<td>81.75</td>
<td>93.59</td>
</tr>
</tbody>
</table>

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

Conflict of interests

The authors claim that there is no conflict of interest.

References


