Chemical constituents of Amygdalus spp. oil from Iran

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Abstract
Background and objectives: Amygdalus with a number of endemic species grow vastly in Iran. Edible sweet almond (A. communis), is cultivated in many countries due to its pharmaceutical, nutritional and cosmetic importance; while almond oil which is rich in oleic acid is known for its economic interest. In the present study, the amount and constituents of oil of six Amygdalus species including A. communis, A. Iranshahrii, A. scoparia, A. lycioides, A. reticulata and A. elaeagnifolia have been analyzed. Methods: The oil of the plants fruits were obtained with hot and cold methods using n-hexane and their percentages were compared. Then, the oils were analysed by GC after methylation of their constituents. Results: The results demonstrated that the hot method gave higher oil yield than cold method. The amounts of the species oils were almost similar except for A. Iranshahrii which contained the least amount of the oil. The most dominant constituents of the oils comprised of palmitic, stearic, oleic and linoleic acids with almost the same pattern, consisting of mostly oleic acid followed by linoleic and palmitic acids. In all samples only little amounts of stearic acid was detectable. Conclusion: Considering the results of the present study, the evaluated species are of high economic value and could be used as alternatives to sweet almond in pharmaceutical, nutritional and cosmetic industries.

Keywords: almond, Amygdalus, fatty acid, fixed oil, GC, oleic acid

Introduction
Diverse plant species grow in Iran, among which some are endemic. Amygdalus (Rosaceae) is a genus with about 20 endemic species growing in different regions of Iran some of which including A. elaeagnifolia, A. scoparia, A. horrida, A. eburnean, A. reticulata, A. Hussknechtii, A. urumiensis, A. glauca and A. lycioides. There are other species which could also be found in north Africa, Balkan islands, south-west Asia, north-east Anatoly, Syria, Iraq, Lebanon, Afghanistan, Turkmenistan and central Asia [1]. Almonds are characteristics of Iran since several species of Amygdalus are endemic to Iran. A. communis (sweet almond), which grows in many parts of the world, is known for its pharmaceutical and nutritional importance. It has
been known by Iranian people since ancient times and has been used in Iranian foods due to its nutritional values. The oil from the seeds has also been used in skin and hair care products. Recent studies have suggested total and LDL cholesterol lowering and HDL increasing effects of almond [2-5]. It has been also shown to have a decreasing effect on anxiety and stress [6]. Almond oil ointments have demonstrated healing effects on skin damages caused by radiotherapy [7]. It has also been shown to have anxiolytic properties [6].

Almond oil which is rich in oleic acid is one of the important export products of Iran [8]. It is known as one of the best and most expensive bases for preparing cosmetics since one optimum goal of preparing these products is moisturizing the skin. Considering that many almond species are endemic to Iran, in the present study, the oil from these species were analysed and compared for better evaluating and estimating their economic importance.

Experimental

Chemicals and reagents

All solvents and reagents were provided from Merck (Germany). The standards including, methyl stearate, methyl palmitate, methyl oleate and methyl linoleate were obtained from Dr. Ehrnestofer GmbH (Germany).

Plant material

The fruits of A. communis, A. Iranshahrii, A. scoparia Spach, A. lycioides Spach, A. reticulata Runemark ex Khatamsaz and A. elaeagnifolia Spach were collected from Fars province, Iran. They were authenticated by Mrs. M. Khatamsaz (botanist).

Preparations

The seeds were separated from the fruits and ground using a hand mill.

Preparing the oils

In order to find the best method for oil extraction, hot and cold extraction methods were compared using sweet almond.

**Hot method**

5 g of the powdered sample was extracted in a 100 mL suxhelet apparatus with n-hexane as solvent. After 6 h, n-hexane was evaporated using a rotary evaporator and the weight of the remaining oil was recorded. The oil preparation was done in triplicate.

**Cold method**

5 g of the powdered sample was macerated in 100 mL n-hexane with continuous shaking. After 24 h, the mixture was filtered and the residue was macerated in the same process for more two times. The n-hexane in the filtrate was evaporated and the weight of the oil was recorded.

Analysis of the oil constituents

In order to analysis of fatty acids in the oils, gas chromatography was used. At first, the fatty acids were methylated by using sodium methylate [9]. The analysis was performed on an Agilent 6890 gas chromatograph, equipped with a CP-Sil 88 capillary column (30 m × 0.25 mm; film thickness 0.25 μm). The oven temperature was programmed at 150-230 °C with rate of 10 °C per min. Then the rate increased to 15 °C per min till 300 °C. Nitrogen was used as the carrier gas at a flow rate of 1 mL/min. Injector temperatures was 240 °C. Detector temperature (FID) was 260 °C. The components were identified by comparing their retention times with standard materials and then their percentage was calculated.

Results and Discussion

Comparing the cold and hot method of oil preparation for sweet almond, it was found that the yields were 1% and 1.83%, respectively. Also the oil from the cold method was turbid while the hot method oil showed a clear appearance. Considering theses points, the hot method was selected for providing the oil from different species of Amygdalus. The results are presented in table 1. As it is shown in table 1, except for A. elaeagnifolia which gave the highest oil content even more than the yield for sweet almond oil,
Yield of the oil obtained from Amygdalus spp.

<table>
<thead>
<tr>
<th>No.</th>
<th>Scientific name</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>A. elaeagnifolia</td>
<td>42.0±3.9</td>
</tr>
<tr>
<td>2.</td>
<td>A. scoparia</td>
<td>34.6±2.2</td>
</tr>
<tr>
<td>3.</td>
<td>A. reticulata</td>
<td>31.2±1.8</td>
</tr>
<tr>
<td>4.</td>
<td>A. lyciciodes</td>
<td>35.2±3.1</td>
</tr>
<tr>
<td>5.</td>
<td>A. communis</td>
<td>36.6±3.5</td>
</tr>
<tr>
<td>6.</td>
<td>A. Iranshahrii</td>
<td>23.6±1.8</td>
</tr>
</tbody>
</table>

The results presented as mean±SD of three experiments.

Other samples demonstrated almost similar yields which was comparable to A. communis. A. Iranshahrii possessed the lowest amount of oil among the tested samples. All species shared similar GC chromatograms. They contained palmitic, stearic, oleic and linoleic acids. Retention times for mentioned acids were 2.94, 3.69, 3.98 and 4.43 min, respectively. The results of fatty acids content of the oils are presented in table 2.

Palmitic acid is a 16C and stearic acid is a 18C saturated fatty acid; while oleic and linoleic acids are 18C unsaturated fatty acids with 1 and 2 unsaturated double bonds, respectively. The amount of palmitic, stearic, oleic and linoleic acids which are present in all species show a similar content pattern with oleic acid comprising the most and stearic acid the least fatty acid in the oils. Linoleic and palmitic acids have the second and third rates in the profile of fatty acids of all almond oils.

Palmitic acid (C_{16}H_{33}O_{2}) is the most common fatty acid among plants and animals. It is the characteristic fatty acid of palm. It is the first fatty acid produced during lypogenesis leading to the production of other fatty acids. Consuming too much of this acid would result in cardiovascular problems [10]; however, its amount is not considerable in plant material and would not be a risk for cardiovascular diseases. Stearic acid (C_{18}H_{36}O_{2}) comprised the least amount in all evaluated oils. The lowest rate was found in A. communis. Stearic acid is found as glyceride in plants and animals. In pharmaceutical industries, it is used as a lubricant, emulsifier and thickening agent in production of soaps, lotions and cosmetics. Compared to other saturated and unsaturated fatty acids, stearic acid causes less raise in cholesterol levels [11]. The highest amounts of oleic acid was found in A. communis; however all species possessed considerable amounts of this fatty acid which was comparable to sweet almond. Oleic acid (C_{18}H_{36}O_{2}), is an unsaturated ω-9 fatty acid found in many plants and animals and is actually the unsaturated form of stearic acid. It is used as emulsifier or co-solvent in aerosols production [8]. Oleic acid has been found to prevent ADL (adrenoleukodystrophy), a fetal disease affecting the brain and adrenal gland, improvement [12]. It has also been found as the reason for the hypotensive potential of olive oil [13]. Since oleic acid was found as the main constituent of all six species, they could be used for the same indications as sweet almond is used. The lowest amounts of linoleic acid were found in A. communis and A. scoparia. Linoleic acid is an essential fatty acid for human and animals which should be obtained from food. It owns a cis double bond and has a role in biosynthesis of arachidonic acid and thus prostaglandins. It is also integrated in the cell membrane. Its deficiency would lead to dermatitis, dry skin, hair loss and delayed wound healing. Consuming

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Palmitic acid (%)</th>
<th>Stearic acid (%)</th>
<th>Oleic acid (%)</th>
<th>Linoleic acid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. communis</td>
<td>6.98±0.37</td>
<td>0.09±0.006</td>
<td>74.41±0.55</td>
<td>16.61±0.26</td>
</tr>
<tr>
<td>A. elaeagnifolia</td>
<td>6.02±0.20</td>
<td>1.59±0.01</td>
<td>68.70±0.35</td>
<td>21.30±0.29</td>
</tr>
<tr>
<td>A. Iranshahrii</td>
<td>9.60±0.69</td>
<td>2.64±0.10</td>
<td>65.62±1.45</td>
<td>18.46±0.74</td>
</tr>
<tr>
<td>A. lyciciodes</td>
<td>8.07±0.23</td>
<td>2.26±0.11</td>
<td>64.01±0.40</td>
<td>23.82±0.29</td>
</tr>
<tr>
<td>A. reticulata</td>
<td>6.99±0.54</td>
<td>1.92±0.07</td>
<td>68.69±1.23</td>
<td>20.60±0.71</td>
</tr>
<tr>
<td>A. scoparia</td>
<td>9.54±0.51</td>
<td>2.75±0.27</td>
<td>68.55±0.82</td>
<td>16.99±1.05</td>
</tr>
</tbody>
</table>
linoleic acid in diabetic patients with $\Delta_6$ desaturase deficiency, would improve diabetes symptoms [14]. It is used in soap making industries and as emulsifier. Because of its anti-inflammatory, acne improving and moisturizing properties, linoleic acid is abundantly used in cosmetic industries [15,16]. All almond samples (especially A. lycioides) contained linoleic acid and could be considered for their nutritional properties; besides, all of the species could be used in cosmetic industries.

Considering the results of the present study, oils from A. lycioides, A. scoparia, A. Iranshahrri, A. reticulata and A. elaeagnifolia are similar to almond oil and could be used as its substitute. They show similar constituents and have little differences in the amounts. Coming to a conclusion, all tested samples could be used in food, pharmaceutical and cosmetic industries. The results of the present study revealed the importance of wild growing almonds in Iran which could be found in almond forests especially in the Fars province which could be of economic importance.

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Declaration of interest
The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

References
