Fatty Acid and Tocochromanol Patterns of Some Salvia L. species

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In the course of our investigations of new sources of higher plant lipids, seed fatty acid compositions and the tocochromanol contents of Salvia bracteata, S. euphratica var. euphratica, S. aucherii var. canascens, S. cryptantha, S. staminea, S. limbata, S. virgata, S. hypargelia, S. halophylla, S. syriaca and S. cilicica were investigated using GLC and HPLC systems. Some of the species are endemic to Turkey. All the Salvia sp. showed the same pattern of fatty acids. Linoleic, linolenic and oleic acid were found as the abundant components. Tocochromanol derivatives of the seed oil showed differences between Salvia species. γ-Tocopherol was the abundant component in most of the seed oils except of S. cilicica. The total tocopherol contents of the seed oils were determined to be more than the total of tocotrienols.

Key words: Salvia, Chemotaxonomy, Fatty Acids and Tocochromanols

Introduction

The Salvia L. genus comprises 900 species all over the world (Standley and Williams, 1973) and it is represented with 88 species in the flora of Turkey. Anatolia is a major centre for the genus in Asia (Davis, 1970, 1988; Güner et al., 2000). Some of the studied Salvia species from East Anatolia and the Mediterranean area studied in here are endemic to Turkey. The genus has economic i.e. medicinal importance and has rich potential as far as the species number and natural widespread in Turkey is concerned. Studies on the distribution of fatty acids (FAs) of seed oils have been driven by economic and taxonomic interests. Previous knowledge about lipids in the Lamiaceae relates mainly to the discovery of new, economically important oil resources in which a number of species from different genera were analysed (Earle et al., 1959). Chia (Salvia hispanica), Perilla, Lallemanitia, Elsholtzia, Dracocephalum (Labiatae) and some others (Aitzetmüller, 1995) were also investigated and evaluated for use as alternative oilseed crops or renewable resources (Aitzetmüller and Tsevegsüren, 1998). More recently, Velasco and Goffman (1999) and Bağcı et al. (2003, 2004) demonstrated the taxonomic potential and significant distribution of an evaluation of seed fatty acids and tocochromanol for some taxa.

Chia (Salvia hispanica L.), a source of industrial oil for the cosmetics industry and of ω-3 α-linolenic acid for the food industry, is one new crop that could help diversify the local economy (Coates and Ayerza, 1998). The present work describes results of analyses of fatty acid composition and content of nutlet lipids of a number of species from the genus Salvia. The aim was to characterize their fatty acids and tocochromanol to establish the taxonomic value and contribution as the renewable resources of FA patterns of these plant taxa.

Experimental

Plant materials

Following plant seeds were collected from natural habitats from different regions of Turkey: Salvia bracteata Banks & Sol., Afyon-Kütahya, 1000 m, Dirmenci-1387; S. euphratica Montbret & Aucher ex Benth. var. euphratica, Sivas-Gürün, Vural-6275; S. aucherii Benth. var. canascens Boiss. & Heldr., Konya-Ermenek, 1100 m, Vural-6189; S. virgata Jacq., Afyon-Kütahya, 1000 m, Dirmenci-1388; S. cryptantha Montbret & Aucher ex Benth., Afyon-Kütahya, 1000 m, Dirmenci-1384; S. halophylla Hedge, Aksaray-Eskil-Gülyazý, 950 m, Vural-7075; S. syriaca L. Elazığ-Oymağac
Table I. Fatty acid composition of some *Salvia* sp. from Turkey (mean ± SD).

<table>
<thead>
<tr>
<th>Salvia sp.</th>
<th>14:0</th>
<th>16:0</th>
<th>16:1 δ7</th>
<th>16:1 δ9</th>
<th>17:0</th>
<th>18:0</th>
<th>18:1 δ9</th>
<th>18:1 δ11</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. bracteata</em></td>
<td>0.60</td>
<td>3.80</td>
<td>0.04</td>
<td>0.00</td>
<td>0.08</td>
<td>0.04</td>
<td>0.04</td>
<td>2.05</td>
</tr>
<tr>
<td><em>S. euphratica</em></td>
<td>0.03</td>
<td>5.40</td>
<td>0.00</td>
<td>0.00</td>
<td>0.07</td>
<td>0.07</td>
<td>0.04</td>
<td>2.17</td>
</tr>
<tr>
<td>var. <em>euphratica</em></td>
<td>0.00</td>
<td>7.77</td>
<td>0.00</td>
<td>0.00</td>
<td>0.10</td>
<td>0.05</td>
<td>0.07</td>
<td>2.05</td>
</tr>
<tr>
<td><em>S. atheri var. canascens</em></td>
<td>0.08</td>
<td>7.77</td>
<td>0.00</td>
<td>0.00</td>
<td>0.10</td>
<td>0.05</td>
<td>0.07</td>
<td>2.05</td>
</tr>
<tr>
<td><em>S. cryptantha</em></td>
<td>0.05</td>
<td>4.73</td>
<td>0.00</td>
<td>0.00</td>
<td>0.07</td>
<td>0.04</td>
<td>0.07</td>
<td>2.33</td>
</tr>
<tr>
<td><em>S. limbata</em></td>
<td>0.04</td>
<td>4.95</td>
<td>0.00</td>
<td>0.00</td>
<td>0.07</td>
<td>0.05</td>
<td>0.05</td>
<td>2.90</td>
</tr>
<tr>
<td><em>S. hypargelia</em></td>
<td>0.75</td>
<td>7.80</td>
<td>0.10</td>
<td>0.00</td>
<td>0.04</td>
<td>0.07</td>
<td>0.10</td>
<td>2.01</td>
</tr>
<tr>
<td><em>S. halophila</em></td>
<td>0.23</td>
<td>5.03</td>
<td>0.00</td>
<td>0.00</td>
<td>0.06</td>
<td>0.36</td>
<td>0.06</td>
<td>1.22</td>
</tr>
<tr>
<td><em>S. virgata</em></td>
<td>0.03</td>
<td>5.41</td>
<td>0.00</td>
<td>0.00</td>
<td>0.06</td>
<td>0.07</td>
<td>0.07</td>
<td>2.63</td>
</tr>
<tr>
<td><em>S. syriaca</em></td>
<td>0.05</td>
<td>5.70</td>
<td>0.00</td>
<td>0.00</td>
<td>0.10</td>
<td>0.06</td>
<td>0.06</td>
<td>2.11</td>
</tr>
<tr>
<td><em>S. staminea</em></td>
<td>0.11</td>
<td>8.24</td>
<td>0.00</td>
<td>0.00</td>
<td>0.06</td>
<td>0.11</td>
<td>0.03</td>
<td>1.85</td>
</tr>
<tr>
<td><em>S. ciliacea</em></td>
<td>0.57</td>
<td>8.66</td>
<td>0.00</td>
<td>0.00</td>
<td>0.08</td>
<td>0.00</td>
<td>0.00</td>
<td>2.83</td>
</tr>
</tbody>
</table>

TSFA: Total saturated fatty acids; TUFA: total unsaturated fatty acids.

village, 850 m, Bagci-1975; *S. ciliacea* Boiss. & Kotschy, Niğde-Ulukışla-Çifteler, 1050 m, Vural-6882; *S. hypargelia* Fisch. & Mey., Adana-Kamışlı-Hamidiye, 1380 m, Vural-6904. *S. limbata* C. A. Mey. (Tr-59072), Ağrı-Tutak, 1700 m and *S. staminea* Montbret & Aucher ex Benth. (Tr-59107), Van-Hasap, 2800 m, seed samples were obtained from seed bank in Aegean Agricultural Research Institute, Izmir.

**Oil extraction and preparation of fatty acid methyl esters (FAME)**

Impurities were removed from the seeds and the cleaned seeds were ground to powder using a ball mill. Lipids were extracted with heptane in a straight through extractor. The triglycerides were transesterified to methyl esters with potassium hydroxide in methanol according to ISO method 5509 (1989).

**Capillary GLC**

The fatty acid methyl ester composition was determined by using different gas chromatographs: Hewlett-Packard HP5890 (A), HP6890 (B), each equipped with a fused silica WCOT capillary and an Unicam-610 (C) FID detector. The results are confirmed with a HP-5973 N GC-MS in Plant Products and Biotechnology Research Laboratory of Firat University, Biology Department.

**Conditions:**

For (A): Silar 5 CP (50 m × 0.25 mm in diameter, 0.24 μm film thickness); carrier gas: nitrogen; split ratio: 1:50, pressure: 160 kPa; oven temperature: 5 min isothermal at 163 °C, then 163 to 205 °C at 1 °C/min; injection temperature: 230 °C; detector temperature: 260 °C.

For (B): DB-23 (60 m × 0.32 mm in diameter, 0.25 mm film thickness); carrier gas: hydrogen; split ratio: 1:50; pressure: 69 kPa; oven temperature: 1 min isothermal at 80 °C, then 80 to 150 °C at 25 °C/min, then 150 to 240 °C at 3 °C/min, 5 min isothermal; PTV-injection temperature: 80 °C, 12 °C/min to 250 °C, 5 min isothermal; detector temperature: 250 °C.

For (C): BPX-70 (15 m × 0.32 mm); carrier gas: nitrogen; split ratio: 1:40; oven temperature: 3 min isothermal at 80 °C, then 80 to 185 °C at 5 °C/min, then 185 to 220 °C at 3 °C/min.

Data analysis was done with a chromato-integrator D 2500 (Merck-Hitachi) and a Chemstation integration software, respectively. Peak identification was achieved by comparison of relative retention times with those obtained from test mixtures of a known composition on two different columns. All determinations were performed in duplicate and the mean values were obtained.

**Tocochromanol analysis**

Tocochromanols were determined by high-performance liquid chromatography (HPLC) according to the procedure of Balz et al. (1992). An aliquot of a solution of 50 mg oil in 1 ml heptane was injected in an HPLC system via a Rheodyne valve with a sample loop volume of 20 μl. Tocopherols
were separated on a LiChrospher 100 Diol phase, 5 mm particle size (Merck, Darmstadt, Germany). HPLC column (25 cm × 4.6 mm in diameter) with an additional guard column (4 mm × 4 mm in diameter), filled with LiChrospher Si 60, 5 mm particle size. The system was operated with the eluent heptane/tert.-butyl methyl ether (96:4 v/v) and detection was made using a fluorescence detector F-1000 (Merck) at 295 nm excitation wavelength and 330 nm emission wavelength. A D-2500 Chromato Integrator (Merck, Darmstadt) was used for data acquisition and processing. Calibration was done by external standards with α-, β-, γ- and δ-tocopherol (Calbiochem, Bad Soden, Germany). Tocotrienols were calculated with the same response factors as the corresponding tocopherols and plastochromanol-8 was calculated with the response factor as γ-tocopherol (Balz et al., 1992).

Results and Discussion

The fatty acid (FA) composition of total nutlet lipids and tocochromanol contents of some Salvia species naturally growing in Turkey were determined. The results of the fatty acid analyses and the oil yield of the Salvia sp. are shown in Table I; the tocopherol and tocotrienol contents are shown in Table II. The analyses showed no significant qualitative difference in fatty acid composition of the analysed Salvia species (Table I). Salvia virgata was showing the highest and S. cryptantha the lowest oil content (Table I). We found usual fatty acids, from C14 to C24 with their unsaturated forms in the studied taxa. It is possible to say that Salvia species studied in here showed qualitatively uniform FA data. Palmitic acid (16:0) was determined in very small amounts. It was ranged between 3.85 and 8.66%. It not found more variable in these and in the other Salvia sp. reported by Aitzelmüller et al. (2003).

The seed oils of five of the studied Salvia sp. (Salvia bracteata, S. euphratica var. euphratica, S. australis var. canascens, S. cryptantha, S. staminea) amounted 69.2 to 58.5% for linoleic acid (18:2 Δ9,12). The other studied Salvia sp. had ca 22.9 to 44.19% content of this component (Table I). The linolenic acid (18:3 Δ9,12,15) contents of these genera showed very different compositional patterns between species. Whereas some species had lower linolenic acid content than 10%, others ranged from ca 20.8 to 55.5%. The large differences between groups in Salvia sp. are very interesting. Oleic acid had similar concentrations between studied Salvia species (23.1 to 16.8%) except S. virgata (10.1%). Oleic acid was the third abundant and more constant component in the studied taxa.

GLC analysis of the studied Salvia sp. showed that there were three different groups. The first group has high linoleic (> 50%) and very low linolenic acid contents (< 10%), respectively (Salvia bracteata, S. euphratica var. euphratica, S. australis var. canascens, S. cryptantha, S. staminea). The second group is the high in linolenic and low in linoleic acid (S. limbata and S. virgata). The third group (S. hypargelia, S. halophylla, S. syriaca and S. ciliarica) has medium linolenic and linoleic acid contents (Table I). These results give some clues on the infrageneric relationships in the genus Salvia. Poly-saturated (PSFA) and unsaturated fatty acids (PUFAs) were detected in low levels and ei-
cosanoic acid homologues in general were lower than 1% (20:0, 22:0, 24:0 with unsaturated forms) (Table I). Total saturated fatty acid (TSFA) composition of the studied Salvia sp. ranged between 6.79 and 12.4%. Total unsaturated FA (TUSFA) contents of Salvia sp. were found very high, 87.5% (S. aucherii var. canascens) to 92.9% (S. halophylla) except for S. staminea. All studies (Ayerza, 1995; Ferlay et al., 1991; Coates and Ayerza, 1998) suggested that the unsaturated fatty acid (USFA) contents of Salvia oils closely resemble each other and chief components are linoleic, oleic and linolenic acid.

The chemotaxonomic significance of the presence or absence of some unusual FAs like phlomic (Phlomis tuberosa) and labellenic (Leonurus sibiricus, Marrubium vulgare) acid in the Lamioideae is not yet known. It is reported that labellenic (18:2 Δ 5,6), phlomic (20:2 Δ 7,8), lamen-allenic (18:3 Δ 5,6, Δ 16 trans), cis-11-eicosenoic, cis-9-eicosenoic acid are the unusual fatty acids found in different genera of the Labiatae like Lamium and Phlomis (Bagby et al., 1965; Mikolajczak et al., 1967; Aitzetmüller and Tsevegsüren, 1998).

The tocopheranol (tocopherol and tocotrienol) derivatives, α-, β-, γ-, δ-tocopherols and tocotrienols, and 8-plastochnoanol were determined in Salvia sp. oils. α-Tocopherol was detected in all of the studied taxa. This result was reported by Demo et al. (1998) for S. fruticosa and S. pomifera. On the other hand, β-tocopherol was not determined or present in very low amount except for S. ciliicica (43.7%). γ-Tocopherol was the most abundant tocopheranol derivative in most of the seed oils. It had maximum concentration in S. cryptantha, S. syriaca, S. virgata and S. limbata. It was not found in S. ciliicica (Table II). δ-Tocopherol was also found in all of the seed oils at contents lower than 10% except for S. bracteata and S. euphratica var. euphratica. Total tocopherol contents were very high (in general > 90%) compared to total tocotrienol contents (< 50%) in the whole seed oils of Salvia species. β-, γ- and δ-tocotrienols were not found or present lower than one percent except γ-tocotrienol in S. ciliicica (Table II). Plastochnoanol-8 was lower than 4% in all the seed oils. The tocopherol profiles of Salvia species showed varying contents of α-, γ- and δ-tocopherols (Table II). The fatty acid and tocopherol composition of plant seed oils can provide characteristic information in order to confirm phylogenetic and taxonomical relations in the plant kingdom (Aitzetmüller, 1993). The Labiatae has shown 18:3 type FAs as dominant, except Scutelleria L. (Marin et al., 1991) and several plants of the family Labiatae are known to produce highly unsaturated seed oils which contain a range of unusual fatty acids (Bohannon and Kleiman, 1975). Enlarged studies on the genera patterns in this family have been continued with the use of different locations patterns as far as chemotaxonomic relationships are concerned.

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Aitzetmüller K. (1993), Capillary GLC fatty acid fingerprints of seed lipids – A tool in plant chemotaxon-
yomy. HRC – Heidelberg 16, 488.
Aitzetmüller K. (1995), Pflanzliche Öle: Namen von Ölen und Fetten und ihre botanische Herkunft – ve-
Aitzetmüller K. and Tsevegsüren N. (1998), Phlomice acid in Lamioideae seed oils. Lamiales Newsletter
(Kew/London) 6, 13–16.
Aitzetmüller K., Tsevegsüren N., and Vosmann K. (1997), A new allenic fatty acid in Phlomis (La-
miaeae) seed oil. Fette – Lipids 79, 74–78.
Aitzetmüller K., Matthias B., and Friedrich H. (2003), A new database for seed oil fatty acids – The Database
Ayerza R. (1995), Oil content and fatty acid composition of Chia (Salvia hispanica L.) from five Northwestern
locations in Argentina. J. Am. Oil Chem. Soc. 72, 1079.
Bagby M. O., Smith C. R., and Wolf I. A. (1965), Label-
venic acid. A new allenic acid from Leonotis nepetae-
Bağcı E., Bruehl L., Aitzetmüller K., and Altan Y. (2003), A chemotaxonomic approach on the fatty acid
and tocochromanol content of Cannabis sativa L. (Cannabaceae). Turkish J. Bot. 27, 141–147.
Coates W. and Ayerza R. (1998), Commercial pro-
duction of Chia in Northwestern Argentina. J. Am.
Oil Chem. Soc. 75, 1417–1420.
Davis P. H. (1970), Flora of Turkey and the East Aegean
Davis P. H. (1988), Flora of Turkey and the East Aegean
Demo A., Petrakis C., Kefalas P., and Boskou D. (1998),
Nutrient antioxidants in some herbs and Mediterra-
Wissenschaftliche Verlagsges., Stuttgart.
Earle F. R., Melvin E. H., Mason L. H., Van Etten C. H.,
and Wolf I. A. (1959), Search for new industrial oils.
I. Selected oils from 24 plant families. J. Am. Oil
Fatty acid composition of seed oils from spontaneous
species of the Mediterranean south-east area. Oleagi-
neux 48, 91.
Güner A., Ozhatay N., Ekim T., and Başer K. H. C.
(2000), Flora of Turkey and the East Aegean Island,
Vol. 11, Suppl. Edinburgh University Press.
Marin D. P., Sajd V., Kapor S., Tatic B., and Petkovic B.
(1991), Fatty acids of the Saturejoideae, Ajugoichae
and Scutellarioideae (Lamiaceae). Phytochemistry 30,
2979–2982.
Mikolajczak K. L., Rohgers M. F., Smith C. R., and Wolf
I. A. (1967), An octadecatrienoic acid from Lamium
purpureum L. seed oil containing 5,6-allenic- and trans-
Standley P. and Williams L. (1973), Labiatae. Fieldiana
Velasco L. and Goffman F. (1999), Chemotaxonomic sig-
ificance of fatty acids and tocopherols in Boragina-