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SHORT COMMUNICATION

Fatty acid composition and chemotaxonomic evaluation of species of *Stachys*

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The fatty acid composition of the seed oil of 23 *Stachys* taxa was analysed by GC/MS. The main compounds were found to be linoleic (27.1–64.3%), oleic (20.25–48.1%), palmitic (4.3–9.1%), stearic (trace to 5.2%) and 6-octadecynoic (2.2–34.1%) acids. The latter compound could be used as a chemotaxonomic marker of the genus *Stachys*. A cluster analysis was performed for comparison and characterisation of the seed oil from *Stachys* species.

Keywords: *Stachys* species; oleic acid; linoleic acid; palmitic acid; 6-octadecynoic acid; chemotaxonomy

1. Introduction

Stachys L. (Lamiaceae, Lamioideae), one of the largest genera of Lamiaceae, contains about 300 species worldwide and is widely grown in warm regions such as the Mediterranean and South West Asia. In the old world, the species is distributed in two main localities; the biggest distribution is in South and Eastern Anatolia, Caucasus, North West Iran and North Iraq, while the other, smaller distribution, is found in the Balkan Peninsula (Radulovic, Lazarevic, Ristic, & Palic, 2007). The species is also found in North and South America and Southern Africa. It is generally grow in rocky places, particularly on limestone and basic rocks in subtropical and tropical regions of both hemispheres (Bhattacharjee, 1974, 1980; Harley et al., 2004; Piozzi & Bruno, 2009).

The latest taxonomic revisions of the genus *Stachys* were conducted by R. Bhattacharjee for the Flora of Turkey (Bhattacharjee, 1982). Eighty-two species (107 taxa) belonging to 12 subsections, 15 sections and two subgenera are reported (Akçicek, 2010) and, the endemism ratio of total taxa in Turkey was observed as 48% (51 of 107 taxa) (Bhattacharjee, 1982; Daşkın, Yılmaz, & Kaynak, 2009; Davis, Mill & Tan, 1988; Dinç & Doğan, 2006; Dinç & Öztürk, 2008; Gemici & Leblebici, 1998; İlçim, Çenet, & Dadandı, 2008; Özhatay, Kültür, & Aslan, 2009; Sümbül, 1990).

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In this study we have investigated the section *Eriostomum*. The section *Eriostomum* (Hoffmans & Link) Dumort consists of 19 species (30 taxa) (Akçiçek, 2010; Dinç & Öztürk, 2008) and is characterised by flowers with upper corolla lip densely sericeous-tomentose outside, hairs usually longer than lip in the genus *Stachys*. The section is further divided into three subsections based on these morphological properties: subsect. *Germanicae* Bhattacharjee (13 taxa), subsect. *Creticae* Bhattacharjee (12 taxa) and subsect. *Spectabiles* Bhattacharjee (5 taxa) (Bhattacharjee, 1982). Twelve taxa of section *Eriostomum* are endemic to Turkey and endemism ratio of this section 41% (Akçiçek, 2010; Bhattacharjee, 1982; Dinç & Öztürk, 2008).

Fatty acid profiles of eight *Stachys* species, i.e. *Stachys balansae* Boiss. et. Kotschy, *Stachys byzantina* C. Koch, *Stachys alopecuroides* (L.) Benth. (from Swiss), *Stachys lanata* Jacq. (from The Netherlands) *Stachys annua* (L.) L., *Stachys monieri* (Gouan) P.W. Ball (from Romania), *Stachys sylvatica* L. and *Stachys milanii* Petrovic (from Yugoslavia), have been reported previously (Marin et al., 1992; Stojanovic et al., 2007). Additionally, seed oil contents of Lamiaceae plant species including *Stachys* were determined in Russia (Akramova, Umarov, & Markmon, 1968) and the fatty acid compositions of essential oils of *Stachys* species from Serbia, Montenegro and Croatia were reported (Radulovic, Lazarevic, Stjepanovic, & Palic, 2006; Radulovic et al., 2007; Vundac et al., 2006).

In this study, we report the fatty acid composition and chemotaxonomic evaluation of 23 different *Stachys* taxa from Turkey and also provide comparison of some other secondary metabolites such as essential oils. This is the first chemical report on the *Stachys huber-morathii* (1), *Stachys thirkei* (3), *Stachys sericantha* (4), *Stachys huetii* (5), *Stachys spectabilis* (6), *Stachys bayburtensis* (7), *Stachys viticina* (8), *Stachys alpina* ssp. *macrophylla* (9), *Stachys germanica* ssp. *bithynica* (10), *Stachys germanica* ssp. *thymphaea* (11), *Stachys pinetorum* (13), *Stachys cretica* ssp. *cassia* (14), *Stachys cretica* ssp. *garana* (16), *Stachys cretica* ssp. *kutahyensis* (17) *Stachys cretica* ssp. *lesbiaca* (18), *Stachys tmolea* (20), *Stachys thracica* (21) and *Stachys cretica* ssp. *bulgarica* (23).

2. Results and discussion

Light yellow oil extracts were obtained *via* a soxhlet extraction of the seed of *Stachys* from selected flora of Turkey, from which 13 different fatty acid components were determined by GC and GC–MS (Gören, Tümen, Çelik, & Çıkrıkçı, 2008; Yaylı, Kıran, Seymen, Genç, & Küçükislamoğlu, 2001).

The chemical relationship between the various *Stachys* species was evaluated using NTSYSpc software, version 2.2 (Palazzo et al., 2009; Rohlf, 2005). The composition percentages of principal components (palmitic, linoleic, oleic, 6-octadecynoic and stearic acids) were used to determine the chemical relationships of the species. Correlation was selected as described in the literature with arithmetic average (UPGMA) (Owolabi et al., 2009).

The principal fatty acid components were found to be linoleic (27.1–64.3%), oleic (20.2–48.1%), palmitic (4.3–9.1%) and stearic (trace to 5.2%) acids. The most important chemotaxonomic marker of species *Stachys* was determined to be 6-octadecynoic acid (2.2–34.1%) (Table 1).

Table 1. Fatty acid compositions of the species of *Stachys*.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Capric acid methyl ester (10:0) ^a	–	–	–	–	t	t	–	–	t	3.6	t	t	t	t	t	t	t	–	–	–	–	–	t
Lauric acid methyl ester (12:0) ^a	–	1.6	0.5	t	t	t	–	–	t	–	t	–	–	t	–	–	–	–	–	–	–	–	–
Myristic acid methyl ester (14:0) ^a	–	–	–	–	–	t	–	–	–	1.7	0.3	–	t	–	–	–	0.4	–	–	–	–	–	t
Palmitoleic acid methyl ester (16:1) ^a	–	–	–	–	t	t	t	–	–	t	–	0.1	t	t	t	t	0.3	t	–	t	–	0.1	0.1
Palmitic acid methyl ester (16:0) ^a	8.5	6.7	5.6	7.6	3.8	5.1	4.3	9.1	5.1	9.1	6.1	7.6	5.7	5.3	7.0	5.6	5.4	5.6	5.1	3.9	5.0	5.1	4.9
Linoleic acid methyl ester (18:2(<i>n</i> –6)) ^a	46.3	27.1	60.1	52.3	51.3	47.1	42.1	47.8	55.3	29.1	45.7	52.1	59.5	50.3	50.2	56.1	54.1	55.7	55.7	48.6	60.2	64.3	62.9
Linolenic acid methyl ester (18:3(<i>cis</i> -9)) ^a	28.6	29.8	24.2	23.8	40.3	36.4	48.1	31.2	30.6	43.2	19.1	23.1	23.1	31.4	29.1	21.1	22.0	21.1	24.1	33.7	23.1	20.2	23.4
Oleic acid methyl ester (18:1(<i>n</i> –3)) ^a	t	t	–	t	t	–	t	–	t	–	t	1.1	t	–	t	t	t	t	t	t	t	–	–
Linolenic acid methyl ester (18:3(<i>n</i> –3)) ^a	13.5	34.1	6.5	11.2	2.2	5.3	3.1	7.6	5.4	7.0	25.2	15.5	6.0	7.5	7.6	9.7	16.1	15.1	8.0	8.9	9.5	8.3	4.5
6-octadecynoic acid methyl ester ^b	2.6	t	2.4	3.7	1.8	2.2	1.6	3.2	2.4	5.2	t	t	2.5	3.3	3.5	2.5	t	t	3.1	1.9	t	t	2.3
Stearic acid methyl ester (C18:0) ^a	t	–	–	–	–	–	–	–	–	–	t	0.3	0.4	t	–	–	0.3	–	–	–	–	0.7	0.3
11,13-Eicosadienoic acid methyl ester (C20:2) ^a	–	–	–	–	–	–	–	–	–	–	–	t	0.3	t	–	–	–	0.4	0.4	–	–	0.3	0.4
11-Eicosenoic acid methyl ester (C20:1) ^a	t	t	–	t	t	–	t	t	t	–	–	t	t	–	–	2.3	0.3	0.1	2.3	t	–	0.2	–
Eicosanoic acid methyl ester (C20:0)	11.1	8.3	8.5	11.3	5.6	7.3	5.9	12.3	7.5	19.6	6.4	7.6	8.2	8.6	10.5	10.4	22.6	21.2	15.4	12.8	14.8	14.0	9.7
Σ Saturated FA	88.4	91.1	90.8	87.3	93.8	88.8	93.3	86.6	91.3	79.3	91.4	91.5	88.6	89.2	86.9	86.9	76.7	76.8	82.9	84.2	84.1	84.9	88.9
Σ Unsaturated FA	99.5	99.4	99.3	98.6	99.4	96.1	99.2	98.9	98.8	98.9	97.8	99.1	96.8	97.8	97.4	97.3	99.3	98.0	98.3	97.0	98.9	98.9	98.6
Total FA																							

Notes: t, trace; Free fatty acid composition of species **1**: *Stachys huber-morathii* **2**: *S. balansae* ssp. *balansae* **3**: *S. thirkei* **4**: *S. sericantha* **5**: *S. huetii* **6**: *S. spectabilis* **7**: *S. bayburtensis* **8**: *S. vilicina* **9**: *S. alpina* ssp. *macrophylla* **10**: *S. germanica* ssp. *bithynica* **11**: *S. germanica* ssp. *thymphaea* **12**: *S. germanica* ssp. *heldreichii* **13**: *S. pinetorum* **14**: *S. cretica* ssp. *cassia* **15**: *S. cretica* ssp. *symmaea* **16**: *S. cretica* ssp. *garana* **17**: *S. cretica* ssp. *kutahyensis* **18**: *S. cretica* ssp. *lesbiaca* **19**: *S. obliqua* **20**: *S. tmolea* **21**: *S. thracica* **22**: *S. byzantina* **23**: *S. cretica* ssp. *bulgarica*.

^aIdentification via Co-injection and MS data.

^bIdentification via MS data.

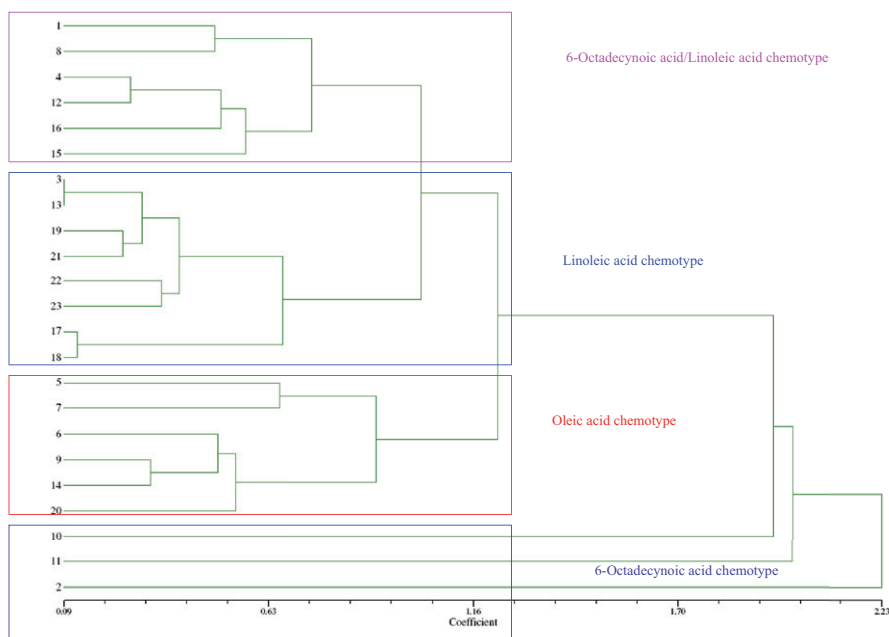


Figure 1. Cluster analysis of the species of *Stachys*.

According to botanical taxonomy, the reported species are divided into three different subsections, depending on their cauline leaves and calyx features (1) *Germanicae*, 1-2-4-9-10-11-12-13-19-20-21, (2) *Creticae*, 3-14-15-16-17-18-22-23 and (3) *Spectabiles*, 5-6-7-8. Using numerical cluster analysis based on fatty acid composition, the relationship of the reported species was divided into four subgroups (Figure 1). For the subsection *Spectabiles*, the main difference from the other species was 6-octadecynoic acid content, with this group containing smaller amounts than the other groups. Particularly, the species 5, 6 and 7 are different, which can easily be observed in the numerical cluster analysis. They are rich in oleic acid content, and are referred to as the oleic acid chemotype (Figure 1). In the subsection *Germanicae*, it is seen that the species 1, 4 and 12 have slightly lower linoleic acid content when compared with the species 3, 15, 16, 22 and 23 from the subsection *Creticae*. The species 1, 4, 8, 12, 15 and 16 are therefore referred to as the linoleic and 6-octadecynoic acid chemotypes. The species 3, 13, 17, 18, 19, 21, 22 and 23 have high linoleic acid contents and, therefore, are the linoleic acid chemotype. The others, 9, 14 and 20, form the oleic acid chemotype together with species 5, 6 and 7. In contrast, species 2, 10 and 11 can be differentiated from the others based on their capric, lauric and myristic acid contents. Then, the species 2, 10 and 11 could be called as 6-octadecynoic acid chemotype.

The species *S. balansae* ssp. *balansae* (2), *S. germanica* ssp. *bithynica* (10), *S. germanica* ssp. *thymphaea* (11) and *S. germanica* ssp. *heldreichii* (12) are the members of *Germanicae*. However, examination of the fatty acid composition of species shows that of these four species, only 2, 10 and 11 have similar

chemical compositions. On the other hand, considering the fatty acid patterns, the species *S. obliqua* (**19**) from the subsect. *Germanicae* and the species *S. byzanthina* (**22**) from the subsect. *Creticae* have been ordered in the same cluster.

It is also important to note that different geographic localities, seasons, harvest periods, properties of soils and climatic conditions strongly affect the secondary metabolite composition of plant species especially essential oil composition.

In our previous study we used a 18:3/18:2 ratio of the seed oil of plant species for chemotaxonomic evaluation (Gören, Kılıç, Dirmenci, & Bilsel, 2006; Gören et al., 2008; Kilic, Dirmenci, & Goren, 2007). However, herein, linolenic acid content of *Stachys* species was determined to be in trace (<0.01%), except *S. germanica* ssp. *thymphaea* **11**, which is 1.1% (Table 1). Hence, this ratio for the further evaluation was not considered. However, the reported species of *Stachys* in this study also have same linolenic and linoleic acid ratios comparable with previous reports in the literature (Marin et al., 1992; Stojanovic et al., 2007). Therefore, this ratio could also be used for chemotaxonomical marker of the *Stachys* species such as other genus from Lamiaceae (Gören et al., 2006, 2008; Kilic et al., 2007; Marin et al., 1992; Stojanovic et al., 2007). Furthermore, apart from linoleic and linolenic acids, the most important chemotaxonomical marker in the genus *Stachys* is 6-octadecynoic acid (Figure 1). The 6-octadecynoic acid (tariric acid) from *S. milanii* has been previously reported and is found to have antifungal activity against fluconazole-resistant *Candida albicans* (Li et al., 2003; Stojanovic et al., 2007).

3. Conclusions

In conclusion, while seed extracts of *Stachys* species have mainly linoleic and oleic acids, linolenic acid content of the genus is seen in a very small amount. The species of *Stachys* have an ability to synthesise unusual fatty acid, 6-octadecynoic acid, which is the main chemotaxonomical marker of species together with oleic and linoleic acids. The species of *Stachys* could be a source of 6-octadecynoic acid. The fatty acid profiles and essential oil compositions of some of the species could also be same chemotaxonomic cluster.

Supplementary material

Experimental details relating to this paper are available online, alongside Table S1.

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