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RESEARCH ARTICLE

Essential oil composition of *Stachys obliqua* Waldst. et Kit.

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Abstract

The genus *Stachys* L. (Lamiaceae) is represented in Turkey by 83 species and altogether 109 taxa. The rate of endemism in Turkey is 43.4 with 33 species. *Stachys. obliqua* Waldst et Kit was collected in July, 2015 in Yarımca to Eskişehir. The essential oil from air-dried aerial parts was isolated by hydrodistillation using a Clevenger apparatus. Chemical composition of the oil was investigated using GC-FID and GC/MS techniques. In total, 97 compounds were identified making up 70.1% of the total volatile constituents. Hexadecanoic acid (10.1%), germacrene D (8.2%), hexahydrofarnesyl acetone (4.4%), β-bourbonene (2.1%) were found as main constituents in the oil.

Keywords: *Stachys obliqua,* essential oil, GC-MS, GC-FID.

Introduction

The genus *Stachys* L., one of the largest genera of the Labiatae (Lamiaceae) family, is widely distributed in the Mediterranean countries, South Western Asia, North and South America and South Africa (Bhattacharjee, 1980). It is represented in Turkey by 83 species and altogether 109 taxa (Akcicek, 2010). The rate of endemism in Turkey is 43.4 with 33 species (Davis et al., 1988, Guner et al., 2000).

Stachys are used as wild tea in Anatolia and Iran (Ozturk et al., 2009) and in Anatolia they are known as 'Adaçayı', `Dağ çayı' and `Balbaşı' (Sezik & Basaran, 1985). Ethno-botanical notes are accessible showing the utilization of species like thyme in Mediterranean cultures (Akcicek et al., 2012, Goren, 2014).

Decoctions or infusions of *Stachys* are utilized as tonics to treat skin disorders or taken internally for gastrointestinal problems (Ozturk et al., 2009), cold, fever and cough (Cakir et al., 1997).

In the literature, some members of the genus have been reported for their anti-inflammatory (Khanavi et al., 2005, Skaltsa et al., 2000), anti anxiety (Rabbani et al., 2003), antibacterial (Grujic-Jovanovic et al., 2004), anti-nephritic (Hayashi et al., 1994), anti-*Helicobacter pylori* (Stamatis et al., 2003) and antioxidant effects (Khanavi et al., 2009).

Our study deals with the analysis of essential oil isolated from the aerial parts of *S. obliqua* Waldst. & Kit growing in Eskişehir, Turkey.

Materials and Methods

Plant material

S. obliqua was collected in July, 2015 in Yarımca to Eskişehir 2nd km by the road side (voucher specimen code: K.H.C. Baser 1867). A voucher specimen is also deposited at the Herbarium of Faculty of Pharmacy of the Anadolu University, Eskişehir, Turkey (ESSE No: 15022).

Isolation of essential oil

The essential oil from air-dried aerial parts was isolated by hydrodistillation using a Clevenger apparatus. Obtained oil was stored in a dark coloured vial at low temperature before analysis. Oil yield was calculated as 0.01% on moisture-free basis.

GC-MS analysis

The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 0.25 μm film thickness) was used with helium as carrier gas (0.8 ml/min). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. Split ratio was adjusted at 40:1. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 35 to 450.

GC analysis

The GC analysis was carried out using an Agilent 6890N GC system. FID detector temperature was 300°C. To obtain the same elution order with GC-MS, simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms. The analysis results are given in Table 1.

Identification of components

Identification of the essential oil components were carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention indices (RRI) to the series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library, MassFinder 3 Library) (McLafferty &Koenig, 1989, Koenig et al., 2004) and in-house "Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data (Joulain & Koenig, 1998, ESO 2000, 1999) was used for the identification.

Results and Discussion

Oil yield was calculated as 0.01% (w/w) from the hydrodistillation of the dried aerial parts of *S. obliqua* using a Clevenger-type apparatus.

In total, 97 compounds were identified by GC and GC/MS analysis, amounting to 70.1% of the whole oil. The composition of the essential oil of *S. obliqua* is reported in Table 2. The chemical composition of *S. obliqua* essential oil showed hexadecanoic acid (10.1%), germacrene D (8.2%), hexahydro farnesyl acetone (4.4%), β-bourbonene (2.1%) as main constituents.

In a previous study, the major components of *S. obliqua* collected from Fethiye were reported as germacrene D (25.4%), thymol (16.4%), borneol (4.9%), α-pinene (4.7%) and isomenthol (3.4%) (Harmandar et al., 1997). According to Goren *et al*. (2011) germacrene D (45%) and β-caryophyllene (17%) were the main components of the oil from plants gathered in Balıkesir.

Table 1. Chemical composition of *S. obliqua* essential oil.

RRI[a]	Compound	%[b]
1032	α-Pinene	tr
1203	Limonene	0.5
1244	2-Pentylfuran	0.1
1280	*p*-cymene	0.1

1393	3-Octanol	0.1
1400	Nonanal	0.8
1443	Dimethyl tetradecane*	0.5
1452	1-Octen-3-ol	1.2
1466	α-Cubebene	0.1
1497	α-Copaene	0.3
1499	α-Campholene aldehyde	0.4
1506	Decanal	0.2
1528	α-Bourbonene	0.3
1535	β-Bourbonene	2.1
1553	Linalool	1.4
1562	Octanol	0.2
1589	β-Ylangene	0.8
1597	β-Copaene	0.6
1600	β-Elemene	0.9
1604	2-Undecanone	0.2
1611	Terpinene-4-ol	0.2
1612	β-Caryophyllene	1.0
1638	β-Cyclocitral	0.3
1648	Myrtanal	0.4
1655	(*E*)-2-Decenal	0.8
1659	γ-Gurjunene	0.6
1670	*trans*-Pinocarveol	0.4
1683	*trans* Verbenol	1.0
1687	α-Humulene	0.2
1688	Selina-4,11-diene	0.5
1694	*p*-Vinyl anisole	0.3
1704	γ-Muurolene	0.3
1706	α-Terpineol	0.7
1715	(*E*,*E*)-2,4-Nonadienal	0.2
1726	Germacrene D	8.2
1740	α-Muurolene	0.3
1744	α-Selinene	0.3
1751	Carvone	0.4
1764	(*E*)-2-Undecenal	0.9
1773	δ-Cadinene	0.6
1776	γ-Cadinene	0.2
1779	(*E*,*Z*)-2,4-Decadienal	0.3
1786	*ar*-Curcumene	0.3
1804	Myrtenol	0.3
1808	Nerol	0.1
1815	2-Tridecanone	0.1
1827	(*E*,*E*)-2,4-Decadienal	0.7
1838	(*E*)-β-Damascenone	0.5
1845	*trans*-Carveol	0.5
1849	Calamenene	0.2
1857	Geraniol	0.2
1868	(*E*)-Geranyl acetone	1.4
1878	1-Methyl naphthalene	0.4

1925	2-Methyl naphthalene	0.3
1945	1,5-Epoxy salvial-4(14)-ene	0.1
1958	(*E*)-β-Ionone	1.2
1965	2-Ethyl hexanoic acid	0.2
1972	1-Ethyl napthalene	0.3
2000	Eicosane	0.1
2008	Caryophyllene oxide	1.5
2009	*trans*-β-Ionone-5,6-epoxide	0.3
2037	Salvial-4(14)-en-1-one	0.3
2041	Pentadecanal	0.7
2046	Norbourbonone	0.3
2050	(*E*)-Nerolidol	0.2
2084	Octanoic acid	0.2
2130	Salviadienol	0.7
2131	Hexahydrofarnesyl acetone	4.4
2145	Valeranone	0.4
2174	Fokienol	0.5
2179	3,4-Dimethyl-5-pentylidene-2(5H)-furanone	1.5
2192	Nonanoic acid	0.2
2200	Docosane	0.5
2209	*T*-Muurolol	0.4
2226	Methyl hexadecanoate	0.3
2255	α-Cadinol	0.5
2273	Selin-11-en-4α-ol	1.5
2278	Torilenol	0.6
2298	Decanoic acid	0.3
2300	Dibenzofuran	0.4
2300	Tricosane	0.4
2324	Caryophylla-2(12),6(13)-dien-5α-ol (=*Caryophylladienol II*)	0.4
2345	Galaxolide-I	0.3
2373	4-oxo-α-Ylangene	0.4
2384	Farnesyl acetone	0.3
2384	1-Hexadecanol	0.3
2500	Pentacosane	0.2
2503	Dodecanoic acid	0.7
2607	1-Octadecanol	1.8
2607	14-Hydroxy-δ-cadinene	0.2
2622	Phytol	0.8
2655	Benzyl benzoate	0.1
2670	Tetradecanoic acid	0.9
2700	Heptacosane	1.4
2740	Anthracene	0.2
2822	Pentadecanoic acid	0.1
2931	Hexadecanoic acid	10.1
	Total	**70.1**

[a]RRI Relative retention indices calculated against *n*-alkanes; % calculated from FID data; tr Trace (< 0.1 %);
* Correct isomer not identified

REFERENCES

Bhattacharjee, B. (1980). Taxonomic studies in *Stachys*: 2. A new infrageneric classification of *Stachys* L. *Notes from the Royal Botanic Garden Edinburgh*, *38*(1), 65-96.

Akcicek, E. (2010). A new subspecies of *Stachys cretica* (section Eriostomum, Lamiaceae) from Turkey. *Turkish Journal of Botany*, *34*(2), 131-136.

Davis, P.H., Mill, R., Tan, K. (Eds.), (1988). Flora of Turkey and East Aegean Islands, Vol. 10. Edinburgh University Press, Edinburgh, pp. 204–206.

Guner, A., Ozhatay, N., Ekim, T., & Baser, K. H. C. (2000). Flora of Turkey and the East Aegean Islands, Vol. 11. *Second Supplement, Edinburgh*.

Ozturk, M., Duru, M. E., Aydogmus-Ozturk, F., Harmandar, M., Mahlicli, M., Kolak, U., & Ulubelen, A. (2009). GC-MS analysis and antimicrobial activity of essential oil of *Stachys cretica* subsp*. smyrnaea*. *Natural product communications*, *4*(1), 109-114.

Sezik, E., & Basaran, A. (1985). Phytochemical investigation on the plants used as folk medicine and herbal tea in Turkey; essential oil of *Stachys lavandulifolia* Vahl. var*. lavandulifolia*. *Journal of Faculty of Pharmacy Istanbul, 21*, 98.

Akcicek, E., Dirmenci, T., & Dundar, E. (2012). Taxonomical notes on *Stachys* sect. Eriostomum (Lamiaceae) in Turkey. *Turkish Journal of Botany*, *36*(3), 217-234.

Goren, A. C. (2014). Use of Stachys species (Mountain Tea) as herbal tea and food. *Records of Natural Products*, *8*(2), 71.

Cakir, A., Duru, M. E., Harmandar, M., Izumi, S., & Hirata, T. (1997). The volatile constituents of *Stachys recta* L. and *Stachys balansae* L. from Turkey. *Flavour and fragrance Journal*, *12*(3), 215-218.

Khanavi, M., Sharifzadeh, M., Hadjiakhoondi, A., & Shafiee, A. (2005). Phytochemical investigation and anti-inflammatory activity of aerial parts of *Stachys byzanthina* C. Koch. *Journal of Ethnopharmacology*, *97*(3), 463-468.

Skaltsa, H., Bermejo, P., Lazari, D., Silvan, A. M., Skaltsounis, A. L., Aurora, S. A. N. Z., & Abad, M. J. (2000). Inhibition of prostaglandin E2 and leukotriene C4 in mouse peritoneal macrophages and thromboxane B2 production in human platelets by flavonoids from *Stachys chrysantha* and *Stachys candida*. *Biological and Pharmaceutical Bulletin*, *23*(1), 47-53.

Rabbani, M., Sajjadi, S. E., & Zarei, H. R. (2003). Anxiolytic effects of *Stachys lavandulifolia* Vahl on the elevated plus-maze model of anxiety in mice. *Journal of Ethnopharmacology*, *89*(2), 271-276.

Grujic-Jovanovic, S., Skaltsa, H. D., Marin, P., & Sokovic, M. (2004). Composition and antibacterial activity of the essential oil of six *Stachys* species from Serbia. *Flavour and Fragrance Journal*, *19*(2), 139-144.

Hayashi, K., Nagamatsu, T., Ito, M., Hattori, T., & Suzuki, Y. (1994). Acteoside, a component of *Stachys sieboldii* MIQ, may be a promising antinephritic agent (2): Effect of acteoside on leukocyte accumulation in the glomeruli of nephritic rats. *The Japanese Journal of Pharmacology*, *66*(1), 47-52.

Stamatis, G., Kyriazopoulos, P., Golegou, S., Basayiannis, A., Skaltsas, S., & Skaltsa, H. (2003). In vitro anti-Helicobacter pylori activity of Greek herbal medicines. *Journal of ethnopharmacology*, *88*(2), 175-179.

Khanavi, M., Hajimahmoodi, M., Cheraghi-Niroomand, M., Kargar, Z., Ajani, Y., Hadjiakhoondi, A., & Oveisi, M. R. (2009). Comparison of the antioxidant activity and total phenolic contents in some *Stachys* species. *African Journal of Biotechnology*, *8*(6).

McLafferty, F.W., Stauffer, D.B. (1989). The Wiley/NBS Registry of Mass Spectral Data, J Wiley and Sons: New York.

Koenig, W. A., Joulain, D., & Hochmuth, D. H. (2004). Terpenoids and related constituents of essential oils. MassFinder 3. *Convenient and Rapid Analysis of GCMS*.

Joulain, D., Koenig, W.A. (1998). The Atlas of Spectra Data of Sesquiterpene Hydrocarbons, EB-Verlag, Hamburg.

ESO 2000 (1999). The complete database of essential oils. The Netherlands: Boelens Aroma Chemical Information Service (BACIS).

Harmandar, M., Duru, M. E., Cakir, A., Hirata, T., & Izumi, S. (1997). Volatile constituents of *Stachys obliqua* L.(Lamiaceae) from Turkey. *Flavour and fragrance Journal*, *12*(3), 211-213.

Goren, A. C., Piozzi, F., Akcicek, E., Kılıç, T., Çarıkçı, S., Mozioğlu, E., & Setzer, W. N. (2011). Essential oil composition of twenty-two *Stachys* species (mountain tea) and their biological activities. *Phytochemistry Letters*, *4*(4), 448-453.