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Chemical Constituents of the Essential Oils of Different Stages of the Growth of *Stachys lavandulifolia* Vahl. From Iran

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Abstract: The essential oil of the aerial parts of different stages of growth as pre-flowering, flowering and post flowering of *Stachys lavandulifolia* Vahl (Lamiaceae) were isolated by hydro distillation. The chemical composition of volatile oil was analyzed by capillary GC and GC/MS. The main components were found to be: α-pinene) 27.25, 25.66, 8.52%), myrcene (17.33, 9.33, 23.85%), β-phellandrene (21.96, 37.49, 12.58%), β-caryophylene (14.3, 8.38, 16.86%).

Key words: *Stachys lavandulifolia*, lamiaceae, essential oil, GC-MS

INTRODUCTION

The subcosmopoltion genus *Stachys* L. comprises more than 270 species (Mabberley, 1997) and is justifiably considered as one of the largest genera of the Labiatae. In the old World area there are two main centres of diversity for the genus, as assessed by the number and distribution of the species. One is confined to South and East Antolia, Caucasia, North West Iran and North Iraq, the other to the Balkan Peninsula (Bhattacharjee, 1980). In Iran, 34 species of the this genus are present, among which, 13 are endemic (Mozaffarian, 1996). The plant is known as Chaye-kuhi in Iran and is a native plant, which has been used as an anxiolytic and sedative in Iranian folk medicine (Amine, 1991). In the present study aerial part of plant at different stage of growth of *S. lavandulifolia* with different chemical composition has been reported.

MATERIALS AND METHODS

Plant material and isolation procedure: The plant material was collected in March (pre-flowering), April (post flowering) and in June (flowering) 2005 from the Zagruse maintain in Lorestan state in south west of Iran. The voucher specimen has been deposited in the Herbarium of the Faculty of Pharmacy, Shaheed Beheshti University of Medical Sciences (herbarium No. 0783).

The oils were obtained by hydro-distillation using a Clevenger-type apparatus for about 2 h and dried over sodium sulfate. The yield of the oils obtained from different stage of growth of *S. lavandulifoila* were pre-f 1.05%, f 1.25%, post-f 1.11%. The oil was analyzed by GC/MS using a Gas Chromatography Analysis GC analysis of the oil was conducted using a Varian CP-3800 instrument equipped with a DB-1 fused silica column (60 m×0.25 mm i.d., film thickness 0.25 μm). Nitrogen was used as the carrier gas at the constant flow of 1.1 mL min^{-1}. The oven temperature was held at 60°C for 1 min, then programmed to 250°C at a rate of 4°C min^{-1} and then held for 10 min. The injector and detector (FID) temperatures were kept at 250 and 280°C, respectively. GC-MS analysis was carried out on a Thermoquest-Finnigan Trace and DB-Wax columns under the same conditions.

GC-MS instrument equipped with a DB-1 fused silica column (60 m×0.25 mm i.d., film thickness 0.25 μm). The oven temperature was raised from 60 to 250°C at a rate of 5°C min^{-1} and then held at 250°C for 10 min; transfer line temperature was 250°C. In this case, the oven temperature was raised from 40 to 250°C at a rate of 4°C min^{-1}, then held at 250°C for 10 min. with the transfer line temperature adjusted at 250°C. Helium was used as the carrier gas at a flow rate of 1.1 mL min^{-1}; split ratio was, 1/50. The quadrupole mass spectrometer was scanned over the 45-465 amu with an ionizing voltage of 70 eV and an ionization current of 150 μA. The constituents of the oil were identified by calculation of their retention indices under temperature-programmed conditions for Identification of individual n-alkanes (C6-24) and the oil on DB-1 compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library (Wiley 7.0) or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those of reported in

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the literature (Davies, 1990; Shibamoto, 1987; Adams, 2001). Quantitative data was obtained from FID area percentages without the use of correction factors. The list of compounds identified in the oil of *S. lavandulifolia* can be seen in Table 1.

RESULTS AND DISCUSSION

Chemical compositions of the hydro-distillated oil is shown in Table 1. In the pre-flowering, flowering and post flowering 41, 37 and 41 compounds were identified, respectively, with major compounds being: α-pinene) 27.25, 25.66 and 28.52%), sabinene (1.69%, 3.29% and 1.07%), β-pinene (2.91, 1.7 and 2.03%), Myrcene (17.33, 9.33 and 23.85%), β-phellandrene (21.96, 37.49 and 12.58%), β-caryophylene (14.3, 8.38 and 16.86%), germacrene-D (3.91, 5.05 and 4.84%), caryophyllene oxide (2.21, 0.08 and 1.03%).

The oil of *S. lavandulifolia* Vahl consisted mainly of monoterpene hydrocarbons (pre-f.: 72.89%, f. : 80.434%, post-f.: 69.42%), oxygenated monoterpenes (pre-f. : 0.36%, f. : 0.68%, post-f. :0.28%), sesquiterpene hydrocarbons (pre-f.: 21.78%, f.: 16.09%, post-f.: 26.64%), oxygenated sesquiterpenes (pre-f.: 3.84%, f.: 1.42%, post-f.: 2.48%), oxygenet diterpene (pre-f.: 0.13%, f.: 0.52%, post-f.: 0.09%). Previous studies on volatile oil of members of the Stachys shows various components.

β-caryophyllene, one of the main components of *S. aleurites* (Flamin *et al*., 2005). α-copaene was detected as the dominate fraction in the oil of *S. byzanthin* (Khanavi *et al*., 2003). α-pinene and β-caryophyllene are the major component of *S. lavandulifolia* Vahl. were collected from Tehran of Iran and Turkey, respectively (Feizbaksh and Tehrani, 2003). In the oils of *S. oblique* (Harmandar *et al*., 1997), *S. laxa* Boiss (sajjadi and Mehresan, 2003), *S. cretica*, *S. scardica*, *S. germanica* (Skaltsa *et al*., 2003), germacrene-D are recorded as the major constituent. In the present study a sample of *S. lavandulifolia* with different chemical composition has been reported monoterpenes were the predominated fraction. In the oils of most *Stachys* species, however, *S. laxa* oil is characterized by a high content of sesquiterpenes (78.6%) with germacrene-D (40.8%) and β-caryophyllene (16.7%) as major components.

At present, it is unknown in which way the composition of volatile oils truly reflects taxonomic relationships in *Stachys*, since many of its members remain to be investigated. However, the chemistry of volatile compounds has been proven particularly helpful in assessing taxonomic relationships of several genera in Labiatae (Skaltsa *et al*., 2003).

Table 1: Composition of the essential oil of Stachys lavandulifolia V

Compound	RI	Area% pre-flowering	Area% flowering	Area% post-flowering
α-thujene	926	0.82	1.34	0.64
α-pinen	936	27.25	25.66	28.52
1-octel-3-ol	962	-	0.06	-
Sabinen	969	1.69	3.29	1.07
β-pinen	977	2.9	1.7	2.03
Myrcene	984	17.33	9.33	23.85
N-decane	999	-	-	0.06
α-phellandrene	1001	0.48	0.87	0.38
α-terpinen	1013	0.05	0.1	0.05
p-cymene	1016	0.07	0.15	0.07
β-phellandrene	1027	21.96	37.49	12.58
Z-β-ocimene	1038	0.18	0.25	0.12
Gama-terpinen	1052	0.11	0.16	0.08
Cis-sabinene hydrate	1058	t	0.11	t
Terpinolene	1081	0.05	-	-
Linalool	1083	0.06	0.16	0.14
Perillene	1088	0.05	t	t
α-capholenal	1109	0.09	0.06	t
Allo-ocimene	1119	0.05	0.09	t
trans-vebinol	1133	0.05	0.08	0.08
Cryptone	1164	-	0.07	t
Terpin-4-ol	1167	0.08	0.23	t
δ-elemene	1339	0.15	0.08	0.22
β-cubebene	1382	0.33	0.27	0.31
β-elemene	1392	0.18	0.26	0.31
β-caryophyllene	1429	14.3	8.38	16.86
Z-β-farnsene	1448	0.06	-	-
E-β-farnsene	1452	0.17	0.13	t
α-humulene	1460	0.21	0.16	t
Germacrene D	1486	3.91	5.05	4.84
Bicyclogermacrene	1501	1.81	1.01	3.39
β-bisabolene	1505	0.05	-	-
δ-cadinene	1522	0.55	0.75	0.57
E-β-bisabolene	1536	0.06	-	0.09
Spathulenol	1575	0.54	0.32	0.84
Caryophyllene oxide	1582	2.21	0.8	1.03
Verdilorol	1593	-	-	0.11
Epiglobulol	1605	0.1	-	0.1
Epi-α-cadinol	1636	0.06	0.08	0.09
α-cadinol	1647	0.06	0.1	0.12
α-Copan-11-ol	1661	-	0.13	-
α-bisabolol	1672	0.8	-	0.06
Leden oxide	1678	0.07	0.12	0.13
Benzyl benzoat	1735	-	-	t
6,10,14-trimethyle-2 pentadecanone	1827	0.06	0.07	-
Plamatic acid	1940	0.07	-	0.24
Phytol	2104	0.13	0.52	0.09

RI = Retaenation time on a DB-1 column in min. Kovat's retation indices as determined on as DB-1 column using the homologous series of n-hydrocarbons. t = trace ($p<0.05\%$)

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