Comparison of the Volatile Composition of *Stachys persica* Gmel. and *Stachys byzantina* C. Koch. Oils Obtained by Hydrodistillation and Steam Distillation

Mahnaz Khanavi[a], Abbas Hadjiakhoondi[a], Gholamreza Amin[a], Yaghoob Amanzadeh[a], Abdolhossein Rustaiyan[c], and Abbas Shafiee[b,*]

[a] Department of Pharmacognosy and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

[b] Department of Chemistry and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran. Fax: +98-21-6461178. E-mail:ashafiee@ams.ac.ir

[c] Department of Chemistry, Sciences and Research Campus, Islamic Azad University, Tehran, Iran

* Author for correspondence and reprint requests

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The oils obtained by hydrodistillation and steam distillation of the aerial parts of *Stachys persica* Gmel. and *Stachys byzantina* C. Koch grown in Iran were analyzed by GC/MS. The essential oil obtained by hydrodistillation of the aerial parts of *S. persica* was characterized by a high amount of non-terpenoid components of which methyllinoleate (27.7%), hexadecanoic acid (9.8%) and 6,10,14-trimethyl-2-pentadecanone (9.2%) were the major constituents, whereas the steam distilled oil of the plant contained hexadecanoic acid (27.2%), carvacrol (9.4%) and eugenol (5.2%).

Both hydrodistilled and steam distilled essential oils of the aerial parts of *S. byzantina* were rich in sesquiterpenes such as α-copaene (16.6% and 10.4%), spathulenol (16.1% and 18.5%) and β-caryophyllene (14.3% and 13.5%), respectively.

Key words: Stachys persica, Stachys byzantina, Essential Oil Composition

Introduction

In Iran, the genus *Stachys* of the Lamiaceae family is represented by 34 species including *S. persica* and *S. byzantina* (Rechinger and Hedge, 1982; Mozaffarian, 1996). This genus has been the subject of a few studies on either oils (Skaltsa *et al.*, 1999, 2001, 2003) or solvent extracts (Maleki *et al.*, 2001; Mantle *et al.*, 2000; Takeda *et al.*, 1997).

Phytochemical analyses of *Stachys* species have confirmed the occurrence of diterpenes (Paternostro *et al.*, 2000; Fazio *et al.*, 1994), phenyl ethanoid glycosides (Miyase *et al.*, 1996; Nishimura *et al.*, 1991), flavonoids (Ansari *et al.*, 1995) and saponines (Yamamoto *et al.*, 1994).

Some species of *Stachys* are used in folk medicine, especially *S. palustris* and *S. sylvatica* (wound wort) which are approved for healing wounds, treating abdominal pains and as disinfectant, antispasmodic and anti-fever (Gruenwald *et al.*, 2000). In Iran, the aerial parts of *S. inflata* are used for infection, asthma, rheumatic and other inflammatory disorders (Maleki *et al.*, 2001).

Many studies have shown various activities in this genus such as anti-inflammatory (Maleki *et al.*, 2001; Skaltsa *et al.*, 2000) and antinephritic effects (Hayashi *et al.*, 1994) as well as an effect on hyaluronidas activity (Takeda *et al.*, 1985) and hypotensive activity (Takeda *et al.*, 1997).

In this paper we report the composition of the essential oil of *Stachys persica* evaluated by hydrodistillation and steam distillation for the first time and the comparison of the components of the steam distilled oil of *Stachys byzantina* with the hydrodistilled oil that we reported before (Khanavi *et al.*, 2003).

Materials and Methods

Plant material

The aerial parts of the two *Stachys* species were collected at Khalkhal, Province of Azarbayjan-Sharghi, Iran in May 1999 (*S. byzantina*) and July 2001 (*S. persica*) during the flowering stage.

Voucher specimens (Number 6528 THE for *S. persica* and 6513 THE for *S. byzantina*, respectively) have been deposited at the Herbarium of the Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

Isolation of the volatile oils

The air-dried aerial parts of both species (100 g) were subjected for 3 h to a separate hydrodistillation using a Clevenger-type apparatus and were also submitted for 1 h to steam distillation.

After decanting and drying of the oils using anhydrous sodium sulfate, the corresponding oils were stored under N_2 in sealed vials until required.

Gas chromatography/mass spectrometry

Water and steam distilled oils of *S. persica* were analyzed by a Hewlett-Packard 5973 mass selective detector connected with a HP 6890 gas chromatograph. The separation was achieved by use of a HP5MS (5% phenylmethylsiloxane) capillary column (60 m × 0.25 mm; film thickness 0.25 μm). The column temperature was held at 60 °C for 3 min and programmed up to 220 °C at a rate of 5 °C/min, and then kept constant at 220 °C for 3 min. Helium was used as the carrier gas (1 ml/min). Mass spectra were taken at 70 eV. Relative percentage amounts were calculated from peak areas using a Shimadzu CR4A chromatopac. GC/MS analysis of the steam distilled oil of *S. byzantina* was performed as it was reported previously (Khanavi *et al.*, 2003).

Identification of the compounds

Retention indices of the components were calculated using retention times of *n*-alkans that were injected after the oil at the same chromatographic conditions. The compounds were identified by comparison of their mass spectra and retention indices (RI) with those reported in the literature (Davies, 1990; Adams, 1995) and of the authentic samples or by comparison with those held in a computer library (Wiley 275.L).

Results and Discussion

Tables I and II show the constituents of the essential oils of *S. persica and S. byzantina.* Both oils were light yellow with a distinct sharp odour in a yielding of 0.3% (w/w) for hydrodistillation and 0.2% (w/w) for steam distillation, respectively.

27 Components were detected in the hydrodistilled oil of *S. persica* representing 93.5% of the total oil. The major constituents were methyllinoleate (27.7%), hexadecanoic acid (9.8%) and 6,10,14-trimethyl-2-pentadecanone (9.2%) where as the steam distilled oil of the plant contained 28 compounds (89.3%), with hexadecanoic acid (27.2%), carvacrol (9.4%) and eugenol (5.2%) as the main constituents.

From Table I, it is evident that the composition of the oils obtained by hydrodistillation and steam distillation of *S. persica* are quantitatively different but the total amount of the non-terpenoid fraction in the hydrodistilled and steam distilled oils of the plant (58.6% and 44.2%) were higher than monoterpenes (11.8% and 22.7%) and sesquiterpenes (23.1% and 22.4%) and some of the identified components in the steam distilled oil were not found in the water distilled oil. Also, oxygenated sesquiterpenes were higher in the steam distilled oil (21.3% against 14.8%). This pattern was observed in oxygenated monoterpenes (22.4% against 11%) too.

In the steam distilled oil of *S. byzantina*, 26 components were identified representing 90.8%, whereas the hydrodistilled oil of the plant contained 24 compounds (88.7%). Both hydrodistilled and steam distilled essential oils of the aerial parts of *S. byzantina*, were rich in sesquiterpenes such as α-copaene (16.6% and 10.4%), spathulenol (16.1% and 18.5%) and β-caryophyllene (14.3% and 13.5%) (Khanavi *et al.*, 2003).

As it is shown in Table II, the differences of the components of water distilled and steam distilled oils of *S. byzantina* are small. Both oils were characterized by high amounts of sesquiterpenes (81.2% and 79.2%), mostly hydrocarbons (55% and 42.8%), but oxygenated sesquiterpenes (36.4% against 26.2%) as well as oxygenated monoterpenes (6.4% against 1.8%) were higher in the steam distilled oil.

Previous investigations on the oil of some species of *Stachys* showed various compositions. Sesquiterpene hydrocarbons were the predominant fraction in the oils of *S. scardica*, *S. cretica* ssp. *cretica*, *S. germanica* ssp. *heldreichii* and *S. laxa* with germacrene D as the major compound (Skaltsa *et al.*, 2003; Sajjadi and Mehregan, 2003) where as spathulenol in *S. spinolosa* and α-copaene and β-caryophyllene in *S. euboica* were the main components (Skaltsa *et al.*, 2003).

Monoterpenes were the major fraction of *S. balansa*, *S. officinalis* and *S. obliqua*, with caryophyllene and germacrene D as sesquiterpene hydrocarbons as the main components (Cakir *et al.*, 1997; Chalchat *et al.*, 2001; Harmandar *et al.*, 1997).

Generally, most species of *Stachys* have rather low amounts of aliphatic and non-terpenoid frac-

Table I. Comparative chemical composition of *Stachys persica* oil obtained by hydrodistillation and steam distillation.

No.	Compound	RI[a]	Hydrodistillation[b] (%)	Steam distillation[b] (%)
1	α-Thujene	931	–	0.3
2	Benzaldehyde	961	1.2	–
3	1-Octen-3-ol	978	4.5	4.2
4	α-Terpinene	1018	0.8	–
5	Linalool	1098	0.8	1.2
6	Nonanal	1100	1.6	0.6
7	Nonanoic acid	1280	–	3.6
8	Thymol	1290	6.6	6.2
9	Carvacrol	1298	2.8	9.4
10	Eugenol	1356	–	5.2
11	β-Cubebene	1390	1.3	–
12	α-Cedrene	1409	–	0.4
13	β-Cedrene	1418	2.3	–
14	Geranyl acetone	1453	0.8	0.4
15	Germacrene D	1480	2.6	–
16	(*E*)-β-Ionone	1485	2.8	3.4
17	δ-Cadinene	1524	2.1	0.7
18	Spathulenol	1576	–	1.6
19	Caryophyllene oxide	1581	4.4	4.9
20	Cedranone	1618	–	0.3
21	Cubenol	1642	0.5	1.5
22	α-Cadinol	1653	1.3	3.4
23	Valeranone	1672	1.4	1.3
24	α-Bisabolole	1683	4.4	4.9
25	Tetradecanoic acid	1772	–	2.1
26	Octadecane	1800	0.2	0.7
27	6,10,14-Trimethyl-2-pentadecanone	1849	9.2	1.6
28	Nonadecane	1900	0.5	0.4
29	Methylhexadecanoate	1927	0.9	0.6
30	Hexadecanoic acid	1978	9.8	27.2
31	Eicosane	2000	0.4	0.3
32	Methyllinoleate	2092	27.7	0.8
33	Linoleic acid	2159	–	2.1
34	Docosane	2200	1.4	–
35	Tricosane	2300	1.2	–
	Total		*93.5*	*89.3*
	Terpenoids			
	Monoterpene hydrocarbons		0.8	0.3
	Oxygenated monoterpenes		11	22.4
	Sesquiterpene hydrocarbons		8.3	1.1
	Oxygenated sesquiterpenes		14.8	21.3
	Non-terpenoides			
	Oxygenated		54.9	42.8
	Non-oxygenated		3.7	1.4

[a] Retention index.
[b] n = 3, the approximate deviation from the mean for hydrodistillation and steam distillation was 5%.

tions where *S. spinolosa* and *S. euboica* have relatively high amounts of fatty acids and aliphatic esters (6.4% and 5.6%) (Skaltsa *et al.*, 2003).

According to our result there were some similarities and differences in chemical composition between these two oil samples and the other oils of *Stachys* species. In addition, in most oils of this genus, the fraction of sesquiterpenes was larger than monoterpenes as the main components of the oils. Also it seems that in steam distilled oils oxygenated terpenoids are higher than in hydrodistilled whereas hydrocarbon terpenoids are the main fraction in hydrodistilled oils of these two species.

Table II. Comparative chemical composition of *Stachys byzantina* oil obtained by hydrodistillation and steam distillation.

No.	Compound	RI[a]	Hydrodistillation[b] (%)	Steam distillation[b] (%)
1	α-Pinene	937	–	1.1
2	Fenchone	1071	0.5	1.0
3	Linalool	1087	0.3	2.1
4	Nonanal	1100	–	0.8
5	Camphor	1126	0.3	0.7
6	α-Terpineol	1189	0.2	0.7
7	Nerol	1213	0.1	0.6
8	Carvone	1218	0.4	0.5
9	α-Cubebene	1354	0.6	0.7
10	α-Copaene	1379	16.6	10.4
11	β-Bourbonene	1380	0.6	0.6
12	β-Cubebene	1387	12.6	7.1
13	β-Caryophyllene	1423	14.3	13.5
14	α-Humulene	1453	8.4	4.5
15	γ-Muurolene	1473	1.2	0.5
16	Epicubebol	1490	0.4	9.3
17	γ-Cadinene	1512	0.4	0.4
18	δ-Cadinene	1518	0.3	5.1
19	Spathulenol	1568	16.1	18.5
20	Caryophyllene oxide	1573	2.8	4.5
21	Humulene epoxide II	1597	6.8	3.4
22	Cubenol	1642	0.1	0.7
23	Eicosane	2000	1.9	1.5
24	Heneicosane	2100	0.9	1.0
25	Tricosane	2300	0.9	0.9
26	Pentacosane	2500	2.0	0.7
	Total		*88.7*	*90.8*
	Terpenoids			
	Monoterpene hydrocarbons		–	1.1
	Oxygenated monoterpenes		1.8	6.4
	Sesquiterpene hydrocarbons		55	42.8
	Oxygenated sesquiterpenes		26.2	36.4
	Non-terpenoids			
	Non-oxygenated		5.7	4.1

[a] Retention index.
[b] n = 3, the approximate deviation from the mean for hydrodistillation and steam distillation was 5%.

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Adams R. P. (1995), Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. Allured Publ. Co., Carol Stream, IL, USA.

Ansari M. A. El., Nawwar M. A., and Saleh N. A. (1995), Stachysetin, a diapigenine-7-glucoside-p-p′-dihydroxy-truxinate from *Stachys aegyptiaca*. Phytochemistry **40**, 1543–1548.

Cakir A., Duru M. E., Harmandar M., Izumi S., and Hirata T. (1997), The volatile constituents of *Stachys recta* L., and *Stachys balansae* L. from Turkey. Flavour Fragr. J. **12**, 215–218.

Chalchat J. C., Petrovic S. D., Makisimovic Z. A., and Gorunovic M. S. (2001), Essential oil of *Stachys officinalis* (L.) Trevis., Lamiaceae from Montenegro. J. Essent. Oil. Res. **13**, 286–287.

Davies N. W. (1990), Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and carbowax 20M phases. J. Chromatogr. **503**, 1–24.

Fazio C., Passannanti S., Paternostro M. P., and Arnold N. A. (1994), Diterpenoids from *Stachys mucronata*. Planta Med. **60**, 499.

Gruenwald J., Brendler T., and Jaenicke C. (2000), PDR for Herbal Medicines. Medical Economics Company, 2nd ed. Montvale, New Jersey, p. 832.

Harmandar M., Duru M. E., Cakir A., Hirata T., and Izumi S. (1997), Volatile constituents of *Stachys obliqua* L. (Lamiaceae) from Turkey. Flavour Fragr. J. **12**, 211–213.

Hayashi K., Nagamatsu T., Ito M., Hattori T., and Suzuki Y. (1994), Acotoside, a component of *Stachys sieboldii* MIQ, may be a promising anti-nephritic agent: effect of acetoside on crescentic-type anti-GBM nephritis in rats. Jpn. J. Pharmacol. **65**, 143–151.

Khanavi M., Hadjiakhoondi A., Shafiee A., Masoudi Sh., and Rustaiyan A. (2003), Chemical composition of the essential oil of *Stachys byzantina* C. Koch from Iran. J. Essent. Oil. Res. **15**, 77–78.

Maleki N., Garjani A., Nazemiyah H., Nilfouroushan N., Eftekhar Sadat A. T., Allameh Z., and Hasannia N. (2001), Potent anti-inflammatory activities of hydroalcoholic extract from aerial parts of *Stachys inflata* on rats. J. Ethnopharm. **75**, 213 –218.

Mantle D., Eddeb F., and Pickering A. T. (2000), Comparison of relative antioxidant activities of British medicinal plant species *in vitro*. J. Ethnopharm. **72**, 497–510.

Miyase T., Yamamoto R., and Ueno A. (1996), Phenylethanoid glycosides from *Stachys officinalis.* Phytochemistry **43**, 475–479.

Mozaffarian V. (1996), A Dictionary of Iranian Plant Names. Farhang Moaser, Tehran, Iran, p. 522.

Nishimura H., Sasaki H., Inagaki N., Chin M., and Mitsuhashi H. (1991), Nine phenethyl alcohol glycosides from *Stachys sieboldii*. Phytochemistry **30**, 965–969.

Paternostro M. P., Maggio A. M., Piozzi F., and Servettaz O. (2000), Labdane diterpenes from *Stachys plumosa*. J. Nat. Prod. **63**, 1166–1167. Rechinger K. H. and Hedge I. C. (1982), Flora Iranica. Akademische Druck- und Verlagsanstalt, Graz, Austria, pp. 150, 360–361.

Sajjadi S. E. and Mehregan I. (2003), Composition of the essential oil of *Stachys laxa* Boiss. & Buhse. Iranian J. Pharm. Res. **1**, 57–58.

Skaltsa H. D., Lazari D. M., Chinou I. B., and Loukis A. E. (1999), Composition and antibacterial activity of the essential oils of *Stachys candida* and *S. chrysantha* from Southern Greece. Planta Med. **65**, 255–256.

Skaltsa H. D., Bermejo P., Lazari D. M., Silvan A. M., Skaltsounis A. L., Sanz A., and Abad M. J. (2000), Inhibition of prostaglandin E2 and leukotriene C4 in mouse peritoneal macrophages and thromboxan B2 production in human platelets by flavonoids from *Stachys chrysantha* and *Stachys candida*. Biol. Pharm. Bull. **23**, 47–53.

Skaltsa H. D., Mavrommati A., and Constantividis T. (2001), A chemotaxonomic investigation of volatile constituents in *Stachys* subsect. Swainsonianeae (Labiatae). Phytochemistry **57**, 235–244.

Skaltsa H. D., Demetzos C., Lazari D., and Sokovic M. (2003), Essential oil analysis and antimicrobial activity of eight *Stachys* species from Greece. Phytochemistry **64**, 743–752.

Takeda Y., Fujita T., Satoh T., and Kakegawa H. (1985), On the glycosidic constituents of *Stachys sieboldii* Miq., and their effects on hyaluronidase activity. Yakugaku Zasshi **105**, 955–959 (in Japanese).

Takeda Y., Zhang H., Masuda T., Honda G., Otsuka H., Sezik E., Yesielada E., and Sun H. (1997), Megastigmane glucosides from *Stachys byzantina*. Phytochemistry **44**, 1335–1337.

Yamamoto R., Miyase T., and Ueno A. (1994), Stachyssaponins I–VIII, New oleanane-type triterpene saponins from *Stachys riederi* CHAMISSO. Chem. Pharm. Bull. **42**, 1291–1296.