Essential Oils in Some Stachys Species growing in Hungary

E. Radnai, Á. Dobos, K. Veres, L. Tóth
Institute of Pharmacognosy, University of Szeged, H-6720 Eötvös str. 6. Hungary,

I. Máthé, G. Janicsák
Institute of Ecology and Botany of the Hung. Acad. Sci., Vácrátót, H-2163 Alkotmány str. 2. Hungary

G. Blunden
School of Pharmacy and Biomedical Sciences, University of Portsmouth, Portsmouth, U.K.

Keywords: Lamiaceae, Stachys, Betonica chemotaxonomy, monoterpenes, sesquiterpenes, iridoids, secondary metabolites

Abstract

The volatile oil compositions of five Stachys species (*Stachys officinalis* L., *St. grandiflora* Host., *St. byzantina* Koch., *St. germanica* L. and *St. sylvatica* L.) have been studied using a GC/MS technique. All species gave low yields of oil. From *St. officinalis* 38 compounds were separated and partly identified, from *St. grandiflora* 24, *St. byzantina* 30, *St. germanica* 29 and *St. sylvatica* 43. All the samples contained o-ocymene, β-phellandrene, γ-terpinene, linalool, trans- and cis-pinocamphone, β-caryophyllene, δ-cadinene and α-cadinol. The steam distilled oils were poor in mono- but rich in sesquiterpenes. Some of the sesquiterpenes, like germacrene D, epi-cubebol, and α- and γ-muurolene are reported in Stachys species for the first time. The iridoids, harpagide and 8-O-acetyl-harpagide, were identified by TLC in the MeOH extracts of *St. sylvatica, St. byzantina, St. officinalis* (*Betonica officinalis*) and *Betonica serotina*. Taking other chemical constituents also into account Stachys species seem to be similar to those of the subfam. Lamioideae (according to Erdtman's classification).

INTRODUCTION

In the course of our systematic chemotaxonomic investigation of the family Lamiaceae (Máthé et al., 1993), we have found the genus Stachys especially interesting for detailed study not only as it is the second largest genus of the family, but also as its chemistry is little known. There exist some uncertainties both in the place of the genus in the family and in the distinction between the genera Stachys and Betonica.

The family Lamiaceae has been classified in different ways by Briquet, Bentham, Erdtman, and Cantino and Sanders (Cantino and Sanders, 1986) and, more recently by Cantino, Harley and Wagstaff (1992). Unlike Erdtman's system, Bentham and Briquet, in their eight subfamilial (Bentham named 'tribes') system, regard the genus Stachys as a member of the subfam. Stachyoideae (tribe Stachydeae in Bentham's system), which has morphological features characteristic of the subfam. Lamioideae in Erdtman's two subfamilial system. On the basis of literature, Stachys species contain iridoids and caffeic acid, lack rosmarinic acid, but yield essential oils. So, from chemical viewpoints, this genus may be a member of Erdtman's subfam. Lamioideae (Cantino and Sanders, 1986, Máthé, et al., 1993, Hegnauer, 1996,1990). The genus also shows some characteristics with regard to flavonoid contents (Kotsos, 2000; Skaltsa, 2000; Barberan, 1988; Skaltsa et al., 1999; Adams 1995).

To verify some of these points, five Stachys species, either native to or easily cultivated in Hungary, have been studied. These are *Stachys officinalis* (L.) Trevis. (*Betonica officinalis* L.), *St. grandiflora* Host., *St. byzantina* Koch., *St. germanica* L. and *St. sylvatica* L. The rightness of the segregation of Stachys officinalis, as Betonica officinalis, from the genus Stachys is also discussed.

MATERIALS AND METHODS

Plants were cultivated in the experimental field of the Research Institute of Ecology and Botany of the Hungarian Academy of Sciences (at Vácrátót, 40 km north of

Budapest)

Fresh plant material (30 g) was gathered for steam distillation. As the oil content was low, it was distilled into n-hexane as an absorbing medium. The oil composition was measured by GC/FID (HP 5890 Series II, capillary column HP-5 30 m, gradient temperature program: from 60 °C to 210 °C then 210-250 °C, by 3 °C/min and 5 °C/min, resp., carrier gas: N2) and GC/MS (ion trap instrument: Finnigen GCQ, MS detector, column: DB-5MS, 30 m, the other main parameters as above, carrier gas: He). Among others, Kováts indexes were used, for the identification of the compounds (11). Some data on non-volatile components, like ursolic (UA), oleanolic (OA), rosmarinic (RA) and caffeic acids (CA) were also evaluated from a MeOH extract of dried plant material (Janicsák and Máthé, 1998).

Iridoids were extracted from the freshly harvested and separated plant materials. Approx. 2 g of fresh plant, mixed with CaCO3, was extracted with 70 % MeOH in an ultrasonic extractor. Then the mixture was filtered through a layer of Al2O3 and made up to 20 ml. From this solution, 20 μl was examined in the presence of harpagide and 8-O acetyl-harpagide by TLC, using ready-made Kieselgel plates (Merck). The development solvent system, used routinely, was

CH_2Cl_2 - MeOH - H_2O (32 + 11 + 1.6)

The compounds were visualised with anisaldehyde (in H_2SO_4) and measured at 520 nm using a Shimadzu densitometer.

RESULTS AND DISCUSSION

- All the species contained essential oil but, in all cases, in very small amount. From Stachys officinalis (*Betonica officinalis*) 38 compounds were separated, from St. grandiflora 24, *St. byzantina* 30, *St. germanica* 29 and *St. sylvatica* 43 (Table 1).
- The sesquiterpene contents were, in all cases, much more than those of the monoterpenes in the oil fractions. With the exception of *St. byzantina*, the monoterpene fractions were between 4.2-6.4 % of the total oil, whereas with St. byzantina, this fraction reached 20 % (Table 1).
- All the samples contained ortho-ocymene, β-phellandrene, γ-terpinene, linalool, trans- and cis-pinocamphone, β-caryophyllene, δ-cadinene, α-cadinol and ethyl hexadecanate (Table 1).
- The monoterpenes detected did not show significant differences from those of Nepetoideae species with high contents of volatile terpenoids.
- Comparing the main iridoid components in the samples obtained from the methanolic extracts of dried drugs, harpagide was found in all the Stachys samples, but not in the Betonica species investigated. In these, only a 'black spot' was detected with the same Rf value as harpagide. However, the Betonica species contained 8-O-acetyl-harpagide, which was not present in the Stachys samples. This difference in the iridoid content suggests that Stachys and Betonica species are different and St. officinalis (*Betonica officinalis* L.) seems to be closer to Betonica serrotina Host. than to the other Stachys species investigated. Our investigations substantiate the opinion of others who regard these three 'species' as one. However, further study of the occurrence of iridoids needs to be undertaken.
- As far as the concentration of iridoids in the organs is concerned, it is an interesting observation that in the stem relatively high amounts can be found, with less in the leaf and, in all cases, the least in the inflorescence. In other iridoid-containing species, like Galium sp. it is on the contrary (Máthé et al., 1988). It is also necessary to carry out more studies about the accumulation pattern before generalisations can be made (Table 2).

If we take into consideration some additional measured parameters, like ursolic/oleanolic acids, rosmarinic and caffeic acids, betaines (glycinebetaine, stachydrine, hydroxystachydrine and trigonelline) and choline (Table 3), it can be concluded that Stachys species are more similar to the species of subfam. Lamioideae than to those of subfam. Nepetoideae. Consequently, our investigations have confirmed

the place of Stachys in the subfam. Lamioideae, as was suggested by Cantino and Sanders (1986) and Cantino, Sanders and Wagstaff (1992).

Literature Cited

Adams, R.P. 1995. Identification of Essential Oil Components by GC/MS, Allured Publishing Corporation, Carol Stream, Illinois, USA.

Barberan, F.A.T., Grayer-Barkmeijer, R.J., Gil, M.I. and Harborne, J.B. 1988. Distribution of 6-hydroxy-, 6-methoxy- and 8-hydroxyflavone glycosides in the Labiatae, the Scrophulariaceae and related families Phytochemistry 27:2631-2645.

Barberan, F.A.T. 1986. The flavonoid compounds from Labiatae. Fitoterapia 57:67-95.

Blunden, G., Yang, M-H., Yuan, Z-X., Smith, B.E., Patel, A.V., Cegarra, J.A., Máthé, I. and Janicsák, G. 1996. Betaine distribution in the Labiatae. Biochem. System. Ecol. 24:1-81.

Cantino, P.G., Harley, R.M. and Wagstaff, S.J. 1992. Genera of Labiatae. Status and Classification. p. 511-522 in R.M. Harley, T.Reynolds (eds.) Advances in Labiate Science. Royal Botanic Gardens Kew.

Cantino, P.D. and Sanders, R.W. 1986. Subfamilial classification of Labiatae. Systematic Botany 11:163-185.

Hegnauer, R. 1966., 1990. Chemotaxonomie der Pflanzen. Vol. IV, IX. Birkhauser Verlag. Basel

Janicsák, G. and Máthé, I. 1998. Parallel determination of rosmarinic and caffeic acids by TLC-densitometry. Chromatographia 46:321-324.

Janicsák, G., Máthé, I., Miklóssy, V.V. and Blunden, G. 1999. Comparative studies of the rosmarinic and caffeic acid contents of Lamiaceae species. Biochemcal Systematics and Ecology 27:733-738.

Kotsos, M.P., Aligiannis, N., Skaltsounis, A.L. and Mitakou, S. 2000. Chemistry of plants from Crete: Stachyspinoside, a new flavonoid glycoside and iridoids from Stachys spinosa. Abstracts of Plenary Lectures, Workshops, Short Lectures and Posters. Natural Products Research in the New Millennium. Int. Cong. and 48th Annual Meeting of the Soc. Med. Plant. Res. (Sept. 3-7, 2000, Zürich) P1A/18

Máthé, I. Jr., Kemertelidze, E.P., Máthé, I. ,Sen., Vadász, Á., Mardaleshvili, T. and Alania, M. 1988. Comparative study of the iridoid production of Hungarian and Georgian Galium verum L. populations. Acta Bot. Hung. 34:257-268.

Máthé, I. Jr., Miklóssy, V.V., Máthé, I., Máthé, Á., Bernáth, J., Oláh, L., Blunden, G. and Patel, A.V. 1993. Essential oil content as chemotaxonomic marker for genus Salvia with reference to its variation in Salvia officinalis L. Acta Horticulturae 330:123-132.

Radnai, E., Janicsák, G., Kocsis, Á., Tóth, L., Dobos, Á. and Máthé, I. Quantitative assay of iriodoids by TLC/densitometry. (in press)

Skaltsa, H., Mavrommati, A., Lazari, D., Tiligada, E. and Constantinidis, Th. 2000. Antimicrobial activity of essential oils of six Stachys taxa belonging to the Sectio Swaisoniana, growing wild in Greece. Abstracts of Plenary Lectures, Workshops, Short Lectures and Posters. Natural Products Research in the New Millennium. Int. Cong. and 48th Annual Meeting of the Soc. Med. Plant. Res., Zürich P2A/56

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 Medica 65:255-256.

Tables

Table 1. Ratios (%) of chemical constituents in steam distillates of Stachys species

A. Monoterpenes

KI	Components	*Stachys officinalis*	*Stachys grandiflora*	*Stachys byzantina*	*Stachys germanica*	*Stachys sylvatica*
		St. off.	St. grand.	St. byz.	St. ger.	St. sylv.
931	α-thujene		tr			
939	α-pinene		tr			
953	camphene	0.30	tr			
967	verbenene	Tr	tr			
976	sabinene	4.69	1.07	12.02	1.48	4.27
980	β-pinene	0.01	0.60	0.08	0.12	
1018	α-terpinene		tr			
1022	o-cymene	tr	0.23	tr	tr	tr
1031	β-phellandrene	tr	0.29	tr	4.76	tr
1040	cis-ocimene	tr	0.23	tr	tr	tr
1043	benzene-acetaldehyde					tr
1050	trans-ocimene	tr	tr	tr	tr	
1062	γ-terpinene	tr	tr	tr	tr	tr
1098	linalool	0.43	0.14	tr	0.03	0.16
1102	α-thujone			tr	0.05	
1140	cis-verbenol					tr
1160	t-pinocamphone	0.33	0.59	4.80	tr	tr
1165	borneol					tr
1173	cis-pinocamphone	0.65	1.08	3.76	tr	tr
1190	methyl salicylate				tr	
1192	dihydro-carveol	tr				tr
1194	myrtenol		tr	0.07		
1200	t-dihydrocarvone			tr		
1298	carvacrol					tr
	Total:	6.41	4.23	20.73	6.44	4.43

Table 1. (continued)Ratios (%) of chemical constituents in steam distillates of Stachys species. B. Sesquiterpenes and other volatile components. .

		St. off.	St.grad.	St.byz.	St. ger.	St. sylv
1351	α-cubebene	tr		tr		tr
1356	eugenol	tr		tr	tr	tr
1372	α-ylangene	tr				tr
1376	α-copaene	2.03		tr	0.11	tr
1384	β-bourbonene	2.77			0.17	0.55
1391	β-elemene	9.98		1.32	0.57	0.88
1418	β-caryophyllene	0.87	5.10	0.21	0.24	1.30
1432	β-gurjunene	tr		0.17	0.27	1.10
1439	α-guainene	tr				
1439	aromadendrene		10.63	0.08		0.45
1454	α-humulene	0.22				
1458	trans-β-farensene				1.98	
1473	γ-gurjunene	0.33				
1477	γ-muurolene			0.16		1.13
1480	γ-curcumene		16.90			tr
1480	germacrene D	3.70		20.99	7.32	33.09
1493	viridiflorene		tr			
1493	epi-cubebol		11.17	0.34		1.31
1495	α-selinene	tr	0.12			1.04
1491	valencene	46.13	7.92		0.06	
1499	α-muurolene			1.98		2.42
1513	γ-cadinene	1.40			tr	tr
1524	δ-cadinene	1.67	1.35	0.35	0.13	2.42
1542	α-calcarone	tr				tr
1556	germacrene B					tr
1563	β-calcarone	tr				tr
1576	spatulenol	0.50		0.23	0.48	1.42
1581	caryophyllene-oxide	0.52			0.57	1.46
1584	β-copaen-4-α-ol	tr				5.80
1590	viridiflorol	tr				1.41
1606	humulene-epoxide	tr	0.67			0.24
1630	α-acorenol					tr
1642	cubenol					tr
1645	α-muurolol	1.47		tr	tr	0.23
1653	α-cadinol	1.49	0.41	0.74	tr	4.03
1672	valerianone			1.51		
1683	α-bisabolol			0.23		
1860	lanceol acetate				tr	
1891	epi-laurenene				0.20	
1927	methyl hexadecanoate			0.72		
1989	manoyloxide					tr
1993	ethyl hexadecanoate	3.15	14.18	17.22	32.73	4.45
2010	epi-13-manoyloxide					tr
2034	kaurene					tr
2056	manool					tr
2302	abietal					tr
	Total:	76.23	68.45	46.25	20.73	64.73
	In all (A+B tables)	82,65	72,52	66,97	61,01	66,73

Table 2. Iridoid content (dry wt %) of some Stachys species (Radnai et al., 2001)

	Leaf		Stem		Inflorescence	
	Harp.	8-O-Ac-Harp.	Harp.	8-O-Ac-Harp.	Harp.	8-O-Ac-Harp.
St. sylvatica	0.17		0.25		0.026	
St. byzantina	0.004		0.02		0.009	
St. officinalis (Betonica officinalis)		0.17		0.25		0.001
Betonica serotina		0.36		0.35		0.096

Harp.= Harpagide; 8-O-Ac-Harp.= 8-O-acetyl-harpagide

Table 3. Some characteristics of the genus Stachys and the subfamilies Lamioideae and Nepetoideae (Máthé et al., 1993; Blunden et al., 1996; Janicsák et al., 1999)

	Genus Stachys	Subfam. Lamioideae	Subfam. Nepetoideae
Ursolic/Oleanolic acids	0.003-0.011	0.003-0.19	0.066-1.43
Rosmarinic acid	0-0.031	0-0.037	0.003-0.062
Caffeic acid	0	0.017-1.07	0
Essential oils	in traces	in traces	rich in many species
Choline	0.004-0.065	0.001-0.11	0.001-0.11
Glycinebetaine	-	0.008-0.084 (6/24)	0.030-0.053 (6/75)
Trigonelline	0.010-0.087	0.004-0.13 (19/24)	0
Stachydrine	traces-1.29	traces-3.22 (19/24)	0
Hydroxy-stachydrine	0.98-1.10	0.21-1.69 (18/24)	0

Figures are % dry wt; in brackets (samples present/samples absent)