Chemistry of Natural Compounds, Vol. 47, No. 5, November, 2011 [Russian original No. 5, September–October, 2011]

ESSENTIAL OIL COMPOSITION OF *Stachys anisochila*

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UDC 547.913

Stachys L. is a genus of about 300 species. It is a subcosmopolitan genus centered in the warm temperate regions of the Mediterranean and SW Asia [1]. Some species of this genus have long been used in folk medicine as sedative, antispasmodic, diuretic, and emmenagogue [2–4]. In the Flora of Serbia, 17 species are recognized [4]. *Stachys anisochila* is Balkan endemic species, distributed in Bulgaria, Serbia, and Albania [5]. It is an erect, densely deflected-hirsute perennial herb which grows on rocky places. The leaves, calyx, and corolla bear nonglandular and glandular trichomes. Previous investigations on the essential oil of members of the *Stachys* genus showed varying composition. The constituents of the essential oil from the numerous *Stachys* species have been studied [6–20]. Many of them possess strong antioxidant and antimicrobial activity [21–24].

Continuing our research on the composition and chemical polymorphism of the essential oil of the genus *Stachys* L., species from Serbia, we now report the results on the volatile oil of *S. anisochila.*

The results of the essential oil analysis of *S. anisochila* are presented in Table 1. GC and GC-MS analysis showed 76 compounds. The main compounds were: α-pinene (7.6%), β-pinene (5.28%), α-copaene (6.25%), and β-caryophyllene (4.48%). *S. anisochila* essential oil contains monoterpenes (34.02%), sesquiterpenes (23.8%), and diterpenes (7.5%). This species, together with *S. menthifolia* and *S. plumosa*, belongs to subgenus *Stachys*, sectio Swainsonniana subsectio Decumbentes [25]. Their essentail oil composition showed that there are remarkable differences in the major constituents.

The essential oil of *S. plumosa* is characterized by high contents of monoterpenes (70%), comprising both α- and β-pinenes as *S. anisochila.* On the other hand, the essential oil of *S. menthifolia* has a very high content of diterpenes (38%). The main components of *S. menthifolia* were abietatriene (12.99%), kaurene (12.35%), valeranone (5%), and α-cadinol (4.2%). The main components of *S. plumosa* were α-pinene (35.84%), β-pinene (31.74%), β-bourbonene (8%), limonene (3.62%), and abietatriene (3.47%).

In our previous work, the essential oil compositions of ten *Stachys* species from Serbia were analyzed. The sesquiterpenes dominated in all the investigated samples of essential oils of *Stachys* species. Monoterpenes were present as well, but in variable amounts. The essential oils of species belonging to the subgenus *Betonica* (*S. officinalis* and *S. scardica*) contain a large proportion of sequiterpenes (70–80%), but the content of monoterpenes is exceptionally low, whereas diterpene compounds are completely lacking.

These results may be useful in chemotaxonomy, but it is necessary to analyze more species of these genera and more populations of each species from different localities, including analyses of other secondary metabolites (e.g., flavonoids).

The essential oils of *Stachys* species are a perspective source of interesting natural compounds. Investigation of the biological activity of essential oils of *Stachys* species showed antibacterial, antifungal, and antioxidative activity, which can be related to the essential oil composition.

Plant Material and Isolation Procedure. The aerial parts of *S. anisochila* were collected at the flowering stage in July 2007, Stara Planina, in Serbia. A voucher specimen has been deposited in the Herbarium of the Institute of Botany and Botanical Garden "Jevremovac," Faculty of Biology, University of Belgrade (BEOU), voucher No. 16349. The aerial parts were air dried in the shade and subjected to hydrodistillation in a Clevenger type apparatus until there was no significant increase in the volume of the oil collected. The oil was eluted with *n*-hexane (Merck), dried over anhydrous sodium sulfate, and stored at 4–6°C.

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0009-3130/11/4705-0823

TABLE 1. Composition of the Essential Oil of *S. anisochila*

Compounds	KI	%	Compounds	KI	%
α-Pinene	936	7.60	N.i.	N.a.	1.11
Camphene	954	0.11	N.i.	N.a.	0.24
β-Pinene	978	5.28	N.i.	N.a.	0.32
α-Terpinene	980	0.70	N.i.	N.a.	0.88
p-Cymene	1018	1.48	Germacrene D	1457	1.80
Limonene	1024	0.25	α-Amorphene	1480	2.57
β-Cymene	1027	2.98	β-Ionone	1485	1.90
γ-Terpinene	1038	1.20	α-Zingiberene	1489	0.20
Terpinolene	1059	1.40	α-Farnesene	1495	0.20
β-Linalool	1085	0.58	δ-Cadinene	1508	0.12
α-Campholenal	1098	1.80	Cadina-1,4-diene	1524	0.20
Pinocarveol	1125	1.80	N.i.	N.a.	0.60
Verbenol	1134	1.60	α-Calacorene	1531	0.41
Pinocarvone	1143	1.98	Nerolidol	1540	0.24
Terpinen-4-ol	1165	2.20	Spathulenol	1564	2.16
Myrtenal	1175	0.90	Caryophyllene oxide	1577	1.12
Myrtenol	1193	1.36	N.i.	N.a.	0.20
Decanal	1194	0.80	N.i.	N.a.	1.10
Bornyl acetate	1203	0.16	α-Cadinol	1581	4.48
N.i.*	N.a.**	0.91	α-Muurolol	1637	1.49
N.i.	N.a.	1.42	α-Bisabolol	1646	1.27
Carvacrol	1285	0.90	N.i.	N.a.	0.71
Tridecane	1297	0.73	N.i.	N.a.	0.10
α-Cubebene	1300	0.31	Benzyl benzoate	1682	2.34
N.i.	N.a.	0.60	Manoyl oxide	1762	0.10
α-Copaene	1348	4.56	Abietatriene	1994	0.20
β-Bourbonene	1375	0.58	Phytol	2058	4.54
β-Caryophyllene	1382	6.25	Tricosane	2135	2.76
N.i.	N.a.	0.95	N.i.	N.a.	0.10
N.i.	N.a.	0.45	N.i.	N.a.	0.88
N.i.	N.a.	1.38	N.i.	N.a.	1.20
N.i.	N.a.	0.78	N.i.	N.a.	0.60
α-Bergamotene	1418	1.05	N.i.	N.a.	0.96
N.i.	N.a.	0.12	Pentacosane	2300	0.36
β-Farnesene	1434	0.14	N.i.	N.a.	1.48
Geranyl acetone	1445	0.20	N.i.	N.a.	2.12
β-Farnesene	1453	0.34	N.i.	N.a.	0.89
N.i.	N.a.	1.58	N.i.	N.a.	0.60

*N.i.: not identified. **N.a.: not available.

Identification of the Oil Components. Qualitative and quantitative analyses of the oil were performed using GC and GC/MS. The GC analysis of the oil was carried out on a GC HP-5890 apparatus, equipped with a split-splitless injector, attached to an HP-5 column (25 m × 0.32 mm, 0.52 μm film thickness) and fitted to an FID. Carrier gas flow rate (H_2) was 1 mL/min, split ratio 1:30, injector temperature 250°C, and detector temperature 300°C, while column temperature was linearly programmed from 40–240°C (at a rate of 4°C).

The same analytical conditions were employed for GC/MS analysis, where an HP G 1800C Series II GCD system was used. The transfer line was heated at 260°C. Mass spectra were acquired in EI mode (70 eV), in the *m/e* range 40–400. An HP-5MS column (30 m × 0.25 mm, 0.25 μm film thickness) was used.

Identification of the individual oil components was accomplished by comparison of their retention times with standard substances and by matching mass spectral data with the MS library (Wiley 275.1) using a computer search and literature [26]. For the purpose of quantitative analysis, area percent obtained by FID was used as base.

ACKNOWLEDGMENT

This research was partially supported by the Serbian Ministry of Science, Technologies and Development (Grant No. 173029).

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