Chemistry of Natural Compounds, Vol. 41, No. 2, 2005

ESSENTIAL OIL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF THE LEAVES OF *Stachys schtschegleevii* FROM IRAN

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UDC 547.913+615.37

The composition of the essential oil obtained by hydrodistillation from the leaves of Stachys schtschegleevii *Sosn. before the flowering stage was analyzed by GC and GC-MS. Forty-five compounds representing 98.7% of the total oil were identified, of which α-pinene (36.4%), germacrene-D (18.6%), limonene (8.2%), and piperitone (6.2%) were the major constituents. Furthermore, antibacterial activity of the entire oil and its two main monoterpenes was evaluated against six Gram-positive and Gram-negative bacteria. The oil exhibited moderate activity against the tested bacteria.*

Key words: *Stachys schtschegleevii*, Labiatae, essential oil composition, antibacterial activity, α-pinene, germacrene-D.

The subcosmopolitan genus *Stachys* L., belongs to the family Labiatae and consists of more than 270 species in the world [1]. In the flora of Iran this genus has been divided into 11 sections with 34 species, of which 13 are endemic. *S. schtschegleevii* Sosn., with the common local name *Pulaky*, is one of the suffruticose native aromatic plants of genus *Stachys*, Sect. *Ambleia*, which is distributed in the North-western and Northern provinces of Iran [2]. *S. schtschegleevii* has been of great interest in ethnopharmacology, especially in the folk medicine of East Azerbaijan (Tabriz). The decoction of the leaves of this plant is used as an antibiotic, and to relieve influenza and cold. Plants of this genus have long been applied in folk medicine to treat genital tumors, inflammatory tumors, ulcers, and cough [3]. Maleki et al., showed that the hydroalcoholic extract of the aerial parts of *S. inflata* exhibits potent anti-inflammatory activity in rat [4]. The constituents of the essential oils of some other *Stachys* species from Iran such as *S. ixodes*, *S. pilifera* and *S. acerosa*, *S. lavandulifolia*, *S. byzantina*, and *S. setifera* subsp. *iranica* have already been reported [5–9]. Also, the essential oil composition and the antibacterial activity of six *Stachys* species from Serbia have recently been published [10]. A literature survey revealed that the essential oil composition and antibacterial activity of *S. schtschegleevii* leaves have not been previously investigated; therefore in this paper the chemical composition and antibacterial activity of the oil of *S. schtschegleevii* is reported.

Hydrodistillation of the leaves of *S. schtschegleevii* gave a yellow oil in a yield of 0.2% (w/w), based on the dry weight of the plant. Forty-five components were identified in the oil, accounting for 98.7% of the total oil. The qualitative and quantitative essential oil compositions are presented in Table 1, where the compounds are listed in the order of their elution from Rtx-1 column. The oil of *S. schtschegleevii* is characterized by a high content of α-pinene (36.4%), germacrene-D (18.6%), limonene (8.2%), and piperitone (6.2%) as the major constituents, followed by β-pinene (4.7%), bicyclogermacrene (3.7%), and valencene (2.5%) in appreciable quantities. The monoterpene hydrocarbons with two main compounds, α-pinene and limonene, comprised 55.6% of the total oil and hence was the most predominant fraction of the oil. The oxygenated monoterpenes constitute 7.9% of the oil, with piperitone as the main component. Among the sesquiterpene hydrocarbons that comprises 29.9% of the oil, germacrene-D was found to be the principal compound. The oxygenated sesquiterpene fraction comprises 5.3% of the total oil, of which elemol (1.4%), α-cadinol (1.4%), and spathulenol (1.2%) were in appreciable percentages.

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0009-3130/05/4102-0171

TABLE 1. Chemical Composition of the Essential Oil of *Stachys schtschegleevii*

RI	Compound	%	Identification method	RI	Compound	%	Identification method
926	Tricyclene	0.8	RI, MS	1382	α-Copaene	0.6	RI, MS
935	**α-Pinene**	**36.4**	RI, MS, CoI	1392	β-Elemene	0.7	RI, MS
960	Oct-1-en-3-ol	0.4	RI, MS	1423	β-Cedrene	Tr.	RI, MS
969	Sabinene	0.4	RI, MS	1427	β-Caryophyllene	0.1	RI, MS
976	β-Pinene	4.7	RI, MS, CoI	1435	β-Gurjunene	Tr.	RI, MS
982	Myrcene	1.4	RI, MS	1445	Aromadendrene	0.1	RI, MS
1001	α-Phellandrene	0.6	RI, MS	1454	γ-Gurjunene	0.1	RI, MS
1009	δ-3-Carene	1.1	RI, MS	1472	γ-Curcumene	Tr.	RI, MS
1010	α-Terpinene	0.1	RI, MS	1475	Isoitalicene	1.3	RI, MS
1013	*p*-Cymene	0.1	RI, MS, CoI	1485	**Germacrene-D**	**18.6**	RI, MS, CoI
1025	**Limonene**	**8.2**	RI, MS, CoI	1489	β-Selinene	0.6	RI, MS
1037	(Z)-β-Ocimene	0.6	RI, MS	1496	Valencene	2.5	RI, MS
1051	γ-Terpinene	0.4	RI, MS, CoI	1500	Bicyclogermacrene	3.7	RI, MS
1082	Terpinolene	0.4	RI, MS	1503	β-Bisabolene	0.8	RI, MS
1084	Linalool	0.5	RI, MS, CoI	1509	γ-Cadinene	0.1	RI, MS
1137	Menthone	0.1	RI, MS	1520	δ-Cadinene	0.6	RI, MS
1177	α-Terpineol	Tr.	RI, MS	1540	Elemol	1.4	RI, MS
1220	Pulegone	0.5	RI, MS	1574	Spathulenol	1.1	RI, MS
1233	**Piperitone**	**6.2**	RI, MS, CoI	1597	β-Eudesmol	0.2	RI, MS
1263	Thymol	0.1	RI, MS, CoI	1609	4b-Hydroxygermacra-1(10),5-diene	0.1	RI, MS
1332	Eugenol	0.5	RI, MS, CoI	1635	α-Cadinol	1.4	RI, MS
1339	δ-Elemene	0.1	RI, MS	1647	α-Eudesmol	1.1	RI, MS
1363	Farnesyl acetate	Tr.	RI, MS		**Total identified**	**98.7**	

RI: Retention indices relative to C_6-C_{24} *n*-alkanes on the Rtx-1 column.
MS: Mass spectrum; CoI, coinjection with an authentic sample.
Tr.: trace (< 0.1%).

TABLE 2. Antibacterial Activity of the Oil of *Stachys schtschegleevii* leaves from Iran

Test organisms	Inhibittion zone, mm			
	oil[a]	main compounds[b]		standard[c]
		α-Pinene	Limonene	Ampicillin
Enterococcus faecalis	10	-	8	11
Staphylococcus aureus	15	7	10	13
Staphylococcus epidermidis	12	-	-	12
Escherichia coli	8	10	11	12
Klebsiella pneumoniae	-	-	-	12
Pseudomonas aeruginosa	-	-	-	9.7

[a]Tested at a concentration of 15 ml/disk.
[b]Tested at a concentration of 10 ml/disk.
[c]Tested at a concentration of 10 ml/disk.
(-)Inactive, (7-15) moderately active, (> 15) highly active.

Previous reports of the oils of other *Stachys* species manifested varying compositions. The major components of the oil of *S. ixodes* Boiss. & Hausskn. ex Boiss. (sect. *Aucheriana*) were found to be myrtenyl acetate (48.7%), globulol (13.1%), caryophyllene oxide (5.9%), and spathulenol (5.6%) [5]. The oil of two other species belonging to sect. *Aucheriana* have also been analyzed, and *cis*-chrysanthenyl acetate was found to be the main component of *S. pilifera* Benth. (25.2%) and *S. acerosa* Boiss. (41.0%) [6]. The oil of *S. lavandulifolia* Vahl. was studied and its main components were reported to be α-pinene (20.1%), β-pinene (12.1%), and spathulenol (7.2%) [7]. The oil of *S. byzantina* C. Koch. was analyzed and it was found to be rich in sesquiterpenes such as α-copaene (16.5%), spathulenol (16.1%), β-caryophyllene (14.3%), and β-cubebene (12.6%) [8]. The main constituents identified in the oil of *S. setifera* subsp. *iranica* were pulegone (26.5%), piperitenone oxide (17.4%), and α-terpinyl acetate (11.26%) [9].

Because of the ethnopharmacological uses of the leaves of *S. schtschegleevii* in Iranian folk medicine, we focused our study on the biological activity of the oil of this plant. Table 2 shows the *in-vitro* antibacterial activity of the hydrodistilled oil of *S. schtschegleevii* leaves and its two main monoterpene constituents, and also the inhibition zones formed by a standard positive control. The oil at the concentration used (15 ml) showed moderate activity against the tested Gram-positive bacteria, of which *Staphylococcus aureus* was the most sensitive to the oil than others. No noteworthy activity was detected against the examined Gram-negative bacteria, except for *Escherichia coli*, which was found to be less sensitive to the oil. Similarly, in another study on the antibacterial activity of six *Stachys* species from Serbia the results showed that these oils exhibit better activity against Gram-positive than against Gram-negative bacteria [10]. Furthermore, the results obtained from two main monoterpenes of the oil, α-pinene and limonene, at 10 ml concentration showed moderate antibacterial activity. *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* were resistant at least at the concentrations of the oil used. The antibacterial property of the oil may, in part, be associated with the contribution of the two major monoterpenes of the oil α-pinene and limonene, which have been examined and were found to have antibacterial activity.

EXPERIMENTAL

Plant Material and Isolation Procedure. The leaves of *S. schtschegleevii* were collected from Ahar (East Azerbaijan province) in June 2003 before the flowering stage. A voucher specimen (No. 200364) has been deposited in the Medicinal Plants and Drugs Research Institute Herbarium (MPRIH), Shahid Beheshti University, Tehran, Iran. Air-dried leaves (100 g) were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus. The distillate was isolated and dried over anhydrous sodium sulfate. The oil was stored at 4°C until analysis.

Oil Analysis Procedure. GC analysis of the oil was conducted using a Varian CP-3800 instrument equipped with a CP-Sil5CB fused silica column (25 m × 0.25 mm i.d., film thickness 0.25 mm). Nitrogen was used as a carrier gas at a constant flow of 1.1 ml/min. The oven temperature was held at 60°C for 1 min, then programmed to 250°C at a rate of 4°C /min, and then held for 10 min. The injector and detector (FID) temperatures were kept at 250°C and 280°C, respectively. GC/MS analysis was carried out on a Thermoquest-Finnigan Trace GC/MS instrument equipped with an Rtx-1 fused silica column (60 m × 0.25 mm i.d., film thickness 0.25 mm). The oven temperature was raised from 60°C to 250°C at a rate of 5°C /min, then held at 250°C for 10 min.; transfer line temperature, 250°C. Helium was used as a carrier gas at a flow rate of 1.1 ml/min; split ratio, 1/50. The quadrupole mass spectrometer was scanned over 45–465 amu with an ionizing voltage of 70 eV and an ionization current of 150 mA.

Identification of the Oil Components. The constituents of the volatile oil were identified by calculation of their retention indices under temperature-programmed conditions for *n*-alkanes (C_6-C_{24}) and the oil on the Rtx-1 column under the same conditions. Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those reported in the literature [11, 12]. Quantitative data was obtained from FID area percentages without the use of correction factors.

Antibacterial Activity. The antibacterial activity of the entire oil and its two main monoterpenes, α-pinene and limonene (Merck, Germany), was tested using the disc diffusion method against three Gram-positive bacteria: *Enterococcus faecalis* (ATCC 15753), *Staphylococcus aureus* (ATCC 25923), and *S. epidermidis* (ATCC 12228) and three Gram-negative bacteria: *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 3583), and *Pseudomonas aeruginosa* (ATCC 27852) [13]. Sterile 6 mm discs were impregnated with 15 ml of the oil and 10 ml of α-pinene and limonene and placed on the surface

of the inoculated agar plates with test bacteria (Standard 1 McFarland). The incubation condition was 37°C for 24 h. All experiments were tested in triplicate. Ampicillin was used as a positive standard control.

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