New Constituents of Essential Oil from *Elsholtzia pilosa**

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By means of GC and GCMS 31 constituents of the essential oil of *Elsholtzia pilosa* have been identified, 1.8-cineol representing the main component (50%). The oil composition with respect to its major constituents has been compared with other Elsholtzia species.

In continuation of our programme to analyse the essential oils of *Elsholtzia* spp. [1, 2], we have undertaken a detailed analysis of *Elsholtzia pilosa. E. pilosa* is an erect herb, often branched from the base, its leaves are ovate, 2–6 cm long, coarsely crenate or crenate-serrate, the petioles are about 1 cm long. Flowers in dense flowered cylindric hispid spikes. Bracts subulate, larger than the flowers, ciliate on the margins, strongly nerved in the middle, calix urceolate, hispid. Corolla pink, 2.5 mm long, 1.5 mm across. *E. pilosa* flowers from September to October, and is distributed from Kumaon to Sikkim and in Khasi mountains [3].

The essential oil from *E. pilosa* which grows around Nainital has been already analyzed by Thappa *et al.* [4], and 14 monoterpenes and 3 sesquiterpenes together with 4 unidentified substances were characterized by gas chromatography. We undertook the study from a chemotaxonomic point of view in order to investigate the presence of acyl furans, typical constituents of *Elsholtzia* species (*cf.* [1] and ref. therein). Moreover, in the course of systematic investigations of insecticidal properties of essential oils (see [1, 5–7] and references therein) the oil was tested for toxicity and insecticidal activity with aphids.

* Terpenoids from Elsholtzia Species, III [1].

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Materials and Methods

The plant material was collected from Dhakuri (7000 ft) on the way to the Pindari glacier (Distt. Almora) and was identified by the local botanist. The essential oil was obtained by steam distillation (0.2% yield) using whole fresh plants.

Gas chromatographic analysis

Hewlett-Packard HP 5890A with Shimadzu CR3A, 50 m FSCC SE54, temp. progr. 4 min at 60 °C, 60–260 °C, 3 °C/min, hold. Split 1:100, injector 240 °C, FID detector 260 °C, carrier gas N_2 with 22 cm/sec linear velocity. The determination of Kovats indices, RI, was performed with a temperature program, producing a linear sequence of retention times for the *n*-alkanes C_{10} to C_{18}, and the indices were compared with those of authentic substances determined under identical GC conditions.

Gas chromatography – mass spectrometry

Quadrupole mass spectrometer Finnigan 3200 E with data system 6000, 25 m FSCC SE54, direct coupling, temp. progr. 5 min at 70 °C, 70–240 °C, 6 °C/min, hold. 10 psig He, 70eV EI-spectra, 1 sec/scan.

The mass spectra were compared with those of authentic samples available and recorded under the same GCMS conditions, as well as with spectra from the literature [8, 9] and from previous works [1, 2, 7, 10–13].

Results and Discussion

By use of gas chromatography and mass spectrometry it was revealed that the essential oil of

E. pilosa consisted of one major constituent, 1.8-cineol **10** (50%). In addition, ten monoterpene hydrocarbons $C_{10}H_{16}$ could be identified, of which α-thujene (**1**, 0.07%), camphene (**3**, trace), sabinene (**5**, 0.77%), myrcene (**7**, 0.50%), α-terpinene (**8**, 0.48%) and (*E*)-ocimene (**11**, 0.80%) have been found for the first time in this species. Different to the report of Thappa *et al.* [4] which was based upon gas chromatographic determinations, we could not detect phellandrene and limonene, although the two substances with 4.21 and 10.22%, respectively, comprised major components almost in their analysis. This discrepancy may be due to different populations, stage of development, environmental factors or to the existence of different chemical races of the plants investigated. However, it seems more likely to be a matter of using a low resolving GC system with packed columns with which a differentiation of isomeric compounds like terpenes might become difficult and give wrong results. Two $C_{10}H_{14}$ hydrocarbons, *p*-cymene (**9**, 0.03%) and the rare verbenene (**4**, trace) were present in the oil, the latter frequently found in oils containing $C_{10}H_{16}O$ alcohols like verbenol, pinocarveol or myrcenol [11] (Table I gives the components of the essential oil of *E. pilosa*, the compounds represented quantitatively in percentage values, and the method of identification, thus reflecting the reliability of the analysis).

Among the oxygenated constituents, *trans*-sabinene hydrate (**13**, 0.60%), *trans*-pinocarveol (**16**, 0.50%), *cis*-verbenol (**17**, 0.20%), pinocarvone (**18**, 2.10%), myrcenol (**19**, 1.10%), 1.5-*p*-menthadien-7-ol (**22**, 0.40%), nerol (**23**, 0.05%), thymol (**24**, 4.30%), 4-phenylbut-3-en-2-ol (**26**, 0.20%) and neryl acetate (**28**, 0.30%) were discovered for the first time in *E. pilosa* oil.

Humulene (**32**, 0.30%), aromadendrene (**34**, 0.20%) and γ-cadinene (**37**, 0.90%) were positively identified among six $C_{15}H_{24}$ sesquiterpene hydrocarbons (all identified compounds are recorded in Table I).

No acyl furan derivatives could be detected in our sample of oil from *E. pilosa,* as they are known as characteristic constituents in other *Elsholtzia* species like *e.g. E. cristata, E. oldhami, E. ciliata, E. nipponica* and *E. densa* (see [1, 2] and references therein). Thus, chemotaxonomically this species is closely related with *E. polystracha* [14], *E. polystachya* [15, 16] and less to *E. strobilifera* [2], since the oils of the first two also contain 1.8-cineole as their major component, whereas in *E. strobilifera* oil pinocarvone is the major and cineol the minor component, the ratio of the two compounds is reversed compared with that of *E. pilosa.*

Table I. Composition of the essential oil of *Elsholtzia pilosa.* The compounds are represented quantitatively in percentage values (FID); the retention indices RI were determined with SE54 and compared with authentic chemicals; mass spectra compared with those of authentic substances under the same recording conditions [m[a]] or with literature [m[b]].

No.	Compound	% (FID)	RI (SE54)	Method of identification
1	α-Thujene	0.07	928	i, m[a]
2	α-Pinene	0.38	935	i, m[a]
3	Camphene	trace	949	i, m[a]
4	Verbenene	trace	956	i, m[a]
5	Sabinene	0.77	975	i, m[a]
6	β-Pinene	1.68	979	i, m[a]
7	Myrcene	0.50	992	i, m[a]
8	α-Terpinene	0.48	1019	i, m[a]
9	*p*-Cymene	0.03	1027	i, m[a]
10	1.8-Cineol	49.94	1032	i, m[a]
11	(*E*)-Ocimene	0.80	1049	i, m[a]
12	γ-Terpinene	5.20	1062	i, m[a]
13	*trans*-Sabinene hydrate	0.60	1071	i, m[a]
14	α-Terpinolene	0.06	1091	i, m[a]
15	Linalool	2.30	1102	i, m[a]
16	*trans*-Pinocarveol	0.50	1144	i, m[a]
17	*cis*-Verbenol	0.20	1150	i, m[a]
18	Pinocarvone	2.10	1169	i, m[a]
19	Myrcenol	1.10	1171	m[b]
20	Terpinen-4-ol	0.30	1180	i, m[a]
21	α-Terpineol	0.80	1193	i, m[a]
22	1.5-*p*-Menthadien-7-ol	0.40	1201	i, m[a]
23	Nerol	0.05	1231	m[a]
24	Thymol	4.30	1295	i, m[a]
25	Acetate $M^+ = 196$	0.30	1322	m[b]
26	4-Phenylbut-3-en-2-ol	0.20	1329	m[b]
27	α-Terpinyl acetate	2.20	1355	i, m[a]
28	Neryl acetate	0.30	1366	i, m[a]
29	$C_{15}H_{24}$	0.90	1399	m[b]
30	$C_{15}H_{24}$	0.30	1420	m[b]
31	Caryophyllene	0.60	1425	i, m[a]
32	Humulene	0.30	1459	i, m[a]
33	$M^+ = m/z$ 190	0.30	1468	m[b]
34	Aromadendrene	0.20	1473	m[a]
35	$C_{15}H_{24}$	0.80	1493	m[b]
36	$C_{15}H_{26}O$	0.50	1527	m[b]
37	γ-Cadinene	0.90	1533	i, m[a]
38	$C_{15}H_{24}O$	0.10	1561	m[b]
39	$C_{15}H_{26}O$	0.60	1619	m[b]
40	$M^+ = m/z$ 218	0.60	1968	m[b]

Method of identification: i = retention index (SE54), m[a] = mass spectrum of authentic sample, m[b] = spectrum compared with literature [8, 9].

The essential oil revealed only little insecticidal properties when it was tested with aphids, *Metopolophium dirhodum* (rate of mortality more than 50% [17] with 1% spray application). Applied to the Mexican bean beetle, *Epilachna varifestis* [17], it showed nearly no activity (mortality 13% after 48 h, 800 μg/insect, topically).

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