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Note

Determination of terpenic compounds in the essential oil from *Satureja thymbra* L. growing in Sardinia

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The essential oils from numerous species of the *Satureja* genus have been widely investigated in the last 20 years[1–17]. Detailed studies have been carried out on *S. parvifoglia*[1], *S. odora*[2], *S. hortensis*[3–5], *S. horvatii*[6–8], *S. boliviana*[9], *S. adamovicii*[10] but, most of all, on *S. montana*[11–17], the gastrosedative and digestive properties of which are well known and appreciated in folk medicine, and whose leaves are used to spice some foods[18,19]. The essential oil from *S. montana* is also employed in liquor manufacture[20] and, recently, its antifungal and antimicrobial activities have been pointed out[21].

In the current literature a few references can be found to research on *S. thymbra* growing in Israel[22,23] and Greece[24] but, to our knowledge, data are lacking on the Italian variety, present exclusively near Cagliari in Sardinia.

For this reason, and also in view of possible pharmaceutical applications, we started our investigation aimed, as a first step, at the identification and dosage of the main components of the essential oil from Sardinian *S. thymbra*.

EXPERIMENTAL

Samples and apparatus

Branches, leaves and apeces, collected in May, June and July, that is before, during and after the blossoming season, were steam distilled for *ca.* 3 h until the complete extraction of the oil was achieved. The oil from each sampling was then separated from the water and, after its specific weight had been determined, transferred to a glass bottle and stored in the dark at 4°C until further analysis.

A Carlo Erba Model 4200 gas chromatograph was used, fitted with flame ionization detection (FID), a split–splitless injector and a linear temperature programmer; the chromatograph was connected to a recorder–integrator (Hewlett-Packard 3390 A). Two different fused-silica columns were employed, loaded respectively with Carbowax 20M liquid phase (A) (column dimensions: 25 m × 0.32 mm I.D.), and CP-Sil 5 liquid phase (B) (column dimensions: 25 m × 0.34 mm I.D.). The operating conditions were; for column A, injector, 230°C, detector, 250°C; initial column temperature 55°C for 5 min, raised at 5°C/min to 200°C and held for 5 min; carrier gas, nitrogen; split ratio 1:40; volume injected 0.2 μl; for column B, injector, 230°C; detec-

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tor 250°C; initial column temperature 55°C for 12 min, raised at 5°C/min to 200°C and held for 5 min; carrier gas, nitrogen; split ratio 1:24; volume injected, 0.2 μl.

An Hewlett-Packard Model 5970 A gas chromatograph–mass spectrometer was also used, equipped with a fused-silica column packed with SE-30 liquid phase (C) (column dimensions: 12 m × 0.22 mm I.D.), under the following conditions; injector 230°C; detector 260°C; initial column temperature, 55°C for 12 min, raised at 5°/min to 200°C and held for 3 min; carrier gas, helium; split ratio 1:10; volume injected, 0.1 μl.

Procedure

A stock solution was prepared containing *ca.* 1000 mg/l in diethyl ether of the following compounds: borneol, camphene, carvacrol, β-caryophyllene, carvone, 1,8-cineole, *p*-cymene, limonene, linalool, β-mycrene, α-pinene, β-pinene, α-terpinene, γ-terpinene and thymol. Working solutions for the calibration graph were then obtained by adding 1 ml of the solution containing the internal standard (menthol) to adequate volumes of the stock solution (from 20 to 200 μl) and by diluting to 10 ml in dietyhl ether.

Each sample was injected in triplicate and analyzed according to the operating conditions listed above. The identities of the peaks were confirmed by comparison of their retention times with those of authentic samples on both column A and B and, when possible, by agreement of their mass spectra, obtained by gas chromatography–mass spectrometry (GC–MS) using column C, with those present in the literature. A mass spectrum of γ-terpinene is shown, as an example, in Fig. 1.

The quantitative analysis was performed, as indicated above, using internal standardization.

RESULTS AND DISCUSSION

The same fourteen terpenic compounds were identified either by GC–FID or by GC–MS in the samples from the three different stages of the vegetative life of *S.*

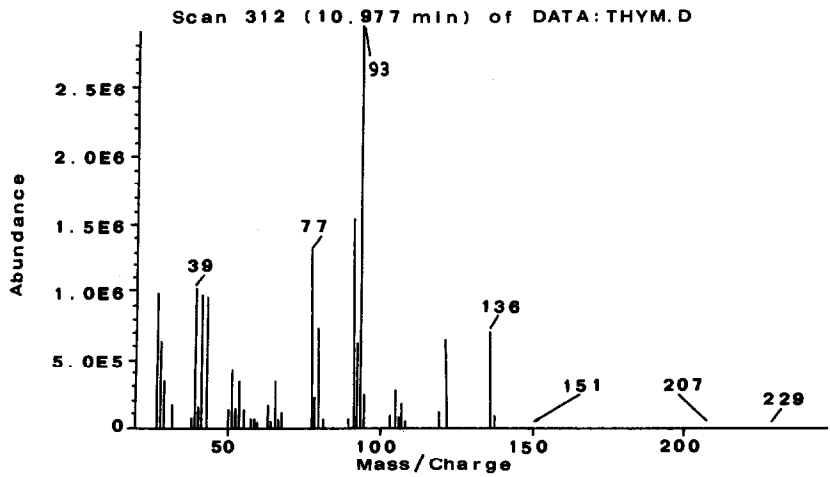

Fig. 1. Mass spectrum of γ-terpinene.

thymbra L. Constituents of the essential oils are listed in Table I according to increasing retention times on column A; this showed the best resolution power, except for limonene and 1,8-cineole which were better resolved on column B.

The concentrations and the absolute percentages (according to the specific weight of each oil) are reported for each terpene. The qualitative composition of the oils appears to be constant in the three stages, but there are notable differences in the percentages of several compounds. γ-Terpinene, thymol and *p*-cymene always show, in this order, the highest concentration and represent, as a whole, from 52.20 to 60.31% of the entire oil.

It must be noted that in all our samples β-caryophyllene was found in considerable amount and, on the other hand, carvone was lacking; however, in GC–MS analysis a compound was detected, with a retention time near to that of carvone and a molecular weight equal to 164, that could be an oxidized form of the same terpene.

Carvacrol, in contrast to reports by numerous authors on other species of the *Satureja* genus[5,68,11,12,15,16] and on *S. thymbra* L.[22–24] was found in low percentages.

The qualitative composition of the essential oil from Israelian *S. thymbra* L[22,23] is similar to the Sardinian one: γ-terpinene, thymol and *p*-cymene are the most abundant constituents and other terpenes, not identified in the essential oil analyzed by us, are present only in traces, like terpinolene, or in very low percentages, like humulene. The same analogy exists, with regard to the main components, between the essential oil from Sardinian *S. thymbra* L. and the Grecian one which differentiates for the lack of β-pinene and limonene and for the presence of other terpenic compounds such as fenchone, cadinene, nerol, geraniol and caryophyllene oxide, though in traces or in very low amounts.

TABLE I

CONCENTRATIONS AND PERCENTAGES OF THE TERPENES IDENTIFIED IN THE ESSENTIAL OIL FROM *S. THYMBRA* L. COLLECTED BEFORE (MAY), DURING (JUNE) AND AFTER (JULY) THE BLOSSOMING SEASON

Peak	*Compound*	*May*		*June*		*July*	
		mg/ml	*%*	*mg/ml*	*%*	*mg/ml*	*%*
1	α-Pinene	19.9	2.38	48.4	5.69	21.5	2.38
2	Camphene	7.2	0.86	15.4	1.81	8.9	1.00
3	β-Pinene	12.2	1.46	28.6	3.36	13.0	1.44
4	β-Myrcene	14.1	1.69	17.8	2.09	15.1	1.68
5	α-Terpinene	24.3	2.92	22.9	2.69	25.5	2.83
6	Limonene	6.7	0.80	9.0	1.06	6.7	0.75
7	1,8-Cineole	3.9	0.47	4.4	0.52	4.3	0.48
8	γ-Terpinene	232.9	27.93	229.6	26.98	264.2	29.39
9	*p*-Cymene	74.6	8.94	76.5	8.99	78.7	8.76
10	Linalool	1.7	0.20	3.7	0.44	1.4	0.15
11	β-Caryophyllene	44.4	5.32	61.9	7.27	22.6	2.52
12	Borneol	9.5	1.14	16.8	1.97	9.9	1.10
13	Thymol	127.9	15.33	157.0	18.45	199.2	22.16
14	Carvacrol	8.6	1.03	16.3	1.92	12.3	1.36

In conclusion the relatively high percentage of β-caryophyllene, the absence of carvone and the low percentage of carvacrol appear to be peculiar and could be useful for the characterization of the essential oil from *S. thymbra* L. growing in Sardinia. Moreover, on the basis of the known biological activity of a number of constituents of the samples of this essential oil, it may be of pharmaceutical use.

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