Chemical Composition of the Essential Oils from *Thymus mastichina* over a Day Period

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Abstract

In the present work, the aerial parts of *T. mastichina*, were collected over a day period, during the flowering phase. The oil was isolated both from the fresh leaves and the fresh flowers by hydrodistillation and analysed by GC and GC/MS. Significant differences were registered between the content and composition of leaf oil and that of flower oil, while changes in consequence of the day period were less emphasised. The highest oil content was obtained from the flowers (2.0 and 2.2 %) at 12 h and 17 h, respectively. The major component of the essential oils was 1,8-cineole, present in higher amounts in the leaf oils than in the flower oils. Its minimal percentages detected in the leaf and the flower oils were 50.2 % and 46.7 %, respectively, observed at 23 h, while the maximal ones were 61.0 % and 50.2 %, at 12 h. Camphor, δ-terpineol + borneol and terpinen-4-ol were also present in higher amounts in the leaf oils than in the flower oils. In both oils, elevated concentrations of δ-terpineol + borneol were detected at 23 h (9.7 % and 8.2 %, respectively). The concentration of myrcene was ten times superior in the flower oils relatively to the leaf oils. In almost all samples, elemol was the sole sesquiterpene whose concentration exceeded 1.0 %.

INTRODUCTION

Thymus mastichina (L.) L. subsp. *mastichina* is an endemic species from the Iberian Peninsula. In Portugal, this plant can be found in dry stony open places, practically all over the country. Spain was one of the major world producers of essential oils from *T. mastichina* of 1,8-cineole type, nevertheless the scattering of this wild plant on irregular and stony fields, the increasing labour cost and the successive droughty years have diminished such production (Garcia Vallejo et al., 1984). These facts suggest the need to cultivate this species under controlled conditions in order to ensure a regular production of the essential oils of high and constant quality. In this study we intended to clear-up the role of plant organ and that of the phenological rhythm during a day on the content and composition of the essential oil in the harvested material.

MATERIAL AND METHODS

Plant Material

For propagation, 5 cm long cuttings, obtained from aerial parts of 15 plants of *T. mastichina* randomly collected at S. Brás de Alportel (Algarve) were placed in rooting pots containing a medium of 75 % non-fertilised peat with 25 % coarse perlite, pH from 5.8 to 6.5. The cuttings were maintained in a greenhouse at a temperature of 10-15 ºC and 50 % air humidity. After 90 days, rooted cuttings were transplanted to 2.5 L pots (three plants each), in sandy soil, and placed under outside conditions. The plant material was fertilised every fifteen days with a solution 1:3:1 (N:P:K) and 0.4 % Mg.

Sampling was done on one day during the flowering period, at 8, 12, 17 and 23 o'clock. After cutting, the flowering shoots were divided into leaf and flower fractions and examined.

Isolation Procedure

The oils were isolated from each sample by hydrodistillation, for 4 hours, using a Clevenger-type apparatus.

Gas Chromatography

The gas chromatographic analyses were performed using a Hewlett Packard 5890 Series II gas chromatograph equipped with a FID, a data handling system and a OV-101 fused silica column (30 m x 0.25 mm; film thickness 0.25 μm). Oven temperature was held at 70 ºC for 5 min and then programmed to 220 ºC at 2 ºC/min. Detector and injector temperatures were set at 260 ºC and 250 ºC, respectively. The carrier gas was helium and the working flow was 1 ml/min. The percentage composition of the oils was computed from the GC peak areas without using correction factors. The data shown are mean values of two injections.

Gas Chromatograph-Mass Spectrometry

The GC/MS analyses were performed using a Perkin Elmer 8320 gas chromatograph, equipped with a DB-5 fused silica column (30 m x 0.25 mm; film thickness 0.25 μm) and interfaced with a Finnigan MAT 800 Ion Trap Detector (ITD; software 4.1). Oven temperature was held at 70 ºC and programmed to 180 ºC at 3 ºC/min. Transfer line temperature, 250 ºC; ion trap temperature, 220 ºC; carrier gas helium adjusted to a linear velocity of 30 cm/s; splitting ratio, 1:100; ionisation energy, 70eV; ionisation current, 60 μA; scan range, 30-400 amu; scan time, 1 s. The identity of the components was assigned by comparison of their retention times and mass spectra with corresponding data of components from reference oils.

RESULTS AND DISCUSSION

The accumulation level of essential oil isolated from *T. mastichina* is given in Table 1. The highest oil yields were always obtained from the flowers, ranging from 1.6 %, at 23 h, to 2.2 %, at 17 h. Larger differences were detected in the levels of leaf oil than in the flower oil. A double amount of leaf oil was obtained in the evening (0.8 % and 0.9 %, at 23 and 17 h, respectively), comparatively to that obtained in the morning (0.4 % both at 8 and 12 h).

The percentages of the main components isolated from the oils of *T. mastichina* are described in Table 2. In quantitative terms, the most important component in the essential oils was 1,8-cineole. The highest percentage registered in the leaf and the flower oils were 61.0 % and 50.2 %, respectively, registered at 12 h. Other oxygen-containing monoterpenes, such as camphor, δ-terpineol + borneol and terpinen-4-ol were also in higher amounts in the leaf oils than in the flower oils. Nevertheless, the largest quantitative differences between leaf and flowers, were detected for camphor. The percentage of this compound in the leaf oil (7.6 - 10.2 %) were almost double when compared to those observed in the flower oil (4.4 - 4.6 %). In contrast, higher levels of linalool and α-terpineol were always present in the flower oils than in the leaf ones. The highest percentages of linalool and α-terpineol in the flower oils were 4.6 % and 4.3 %, while the most elevated concentrations in the leaf oils did not exceed 1.8 % and 2.5 %, respectively.

α-Pinene, camphene, sabinene and β-pinene were the sole monoterpene hydrocarbons whose concentrations exceeded 1.0 % either in the leaf oils or in the flower ones. The flower oils were always richer in α-pinene, sabinene, β-pinene and myrcene, among which the most important difference was observed for myrcene. Six to ten times more myrcene were registered in the flower oil than in the leaf oil, that is, 1.5 % to 1.6 % were detected in the flower oils, whereas in the leaf oil the level ranged from traces to 0.3 %. In contrast, higher levels of *p*-cymene were observed in the leaf oils (1.3 - 1.7 %) than in the flower oils (0.2 - 0.4 %). Among sesquiterpenes, elemol was the only one whose concentration, was higher, than 1.0 % in practically all samples. The highest proportion (2.3 %) of elemol was detected in the flowers at 17 h.

Differences in the oil composition during the day were less important, than that between the organs. An increase in the proportion of 1,8 cineole in the morning time (8-12h) comparing to the later periods was observed both for flowers and leaves. In both the leaf and the flower oils, the most elevated concentrations of δ-terpineol + borneol were detected at 23 h (9.7 % and 8.2 % in leaves and flowers, respectively).

Literature Cited

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Tables

Table 1. Essential oil content of the leaves and the flowers of *T. mastichina* (L.) L. subsp. *mastichina* (% of fresh mass)

Plant organ	Time, during day (h)			
	8:00	12:00	17:00	23:00
Leaf oil content %	0.4	0.4	0.9	0.8
Flower oil content %	1.7	2.0	2.2	1.6

Table 2. Percentages of the main components of the essential oils from the leaves (Lf.) and the flowers (Fl.) of *Thymus mastichina* over a day period

Component		Leaf	Flower	Leaf	Flower	Leaf	Flower	Leaf	Flower
		Time (h) during day							
	RI	8:00		12:00		17:00		23:00	
α-pinene	0925	3.2	5.7	2.4	6.5	3.5	6.4	3.3	5.7
camphene	0940	6.1	4.3	4.4	4.6	5.3	4.6	5.3	5.0
sabinene	0964	1.1	3.4	1.1	3.2	1.7	3.3	1.4	3.4
β-pinene	0971	1.8	4.2	1.6	4.4	2.4	4.3	2.2	4.2
myrcene	0980	T	1.5	0.1	1.6	0.3	1.5	0.3	1.5
α-terpinene	1012	1.3	0.2	0.1	0.7	0.2	0.5	0.2	0.5
p-cymene	1015	1.5	0.4	1.4	0.3	1.3	0.2	1.7	0.3
1,8-cineole	1026	55.7	49.1	61.0	50.2	54.0	47.7	50.2	46.7
trans-β-ocimene	1037	T	1.2	0.1	1.3	0.3	1.5	0.3	1.9
γ-terpinene	1050	0.1	0.5	0.4	1.1	0.5	0.9	0.6	0.8
trans-sabinene hydrate	1055	-	2.9	1.4	1.2	2.4	1.8	1.5	2.8
terpinolene	1081	-	0.2	0.1	0.4	0.2	0.3	0.2	0.3
linalool	1087	0.8	4.6	1.4	4.5	1.8	3.9	1.5	3.0
Camphor	1125	10.1	4.6	8.5	4.2	7.6	4.4	8.5	4.3
δ-terpineol + borneol	1150	7.8	6.0	6.5	5.9	7.4	6.4	9.7	8.2
terpinen-4-ol	1165	3.4	1.1	2.6	1.9	2.1	1.9	3.5	1.5
α-terpineol	1176	1.8	4.0	1.7	4.2	2.5	4.2	2.5	4.3
bornyl acetate	1274	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.1
β-caryophyllene	1426	0.3	0.5	-	-	0.2	0.4	0.2	0.6
elemol	1542	0.3	2.0	0.5	1.2	1.0	2.3	1.0	2.1
caryophyllene oxide	1581	0.4	0.1	0.2	0.1	0.3	0.1	0.3	T
γ-eudesmol	1620	-	0.1	t	0.2	t	0.2	t	0.2
t-cadinol	1628	-	0.2	t	0.2	t	0.2	t	0.1
β-eudesmol	1635	0.5	0.2	0.1	0.2	0.1	0.2	0.1	0.2
intermedeol + α-eudesmol	1651	0.3	1.2	0.5	0.8	0.6	0.8	0.6	0.5

-: not detected
t: traces
RI: Retention Index relative to C_8-C_{21} *n*-alkanes on the OV-101.