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

M.G. Miguel
Unidade de Ciências e Tecnologias Agrárias, University of Algarve
Campus de Gambelas,
8000-117 Faro, Portugal

F. Duarte, F. Venâncio and R. Tavares
INETI, IBQTA, Departamento de Tecnologia das Indústrias Químicas, Estrada do Paço do Lumiar, 1699 Lisboa Codex,
Portugal

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Abstract

In the present work, the aerial parts of *T. mastichina* maintained in pots, on a sandy soil and fertilized every fifteen days with a solution 1:3:1 (N:P:K) and 0.4 % Mg, were collected over a day period, during the flowering phase. The oils were isolated from both the fresh leaves and the fresh flowers by hydrodistillation and analyzed by GC and GC/MS. The highest oil yields were obtained from the flowers, the highest ones (2.0 % and 2.2 %, v/w) being observed when the flowers were collected 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. The 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 in higher amounts in the leaf oils than in the flower oils. In both the leaf and the flower oils, more elevated concentrations of δ-terpineol+borneol were detected at 23 h (9.7 % and 8.2 %, respectively). α-Pinene, camphene, sabinene and β-pinene were the monoterpene hydrocarbons whose concentrations exceeded 1.0 %. The flower oils were richer in α-pinene, sabinene, β-pinene and myrcene than the leaf oils. The most important quantitative difference was observed for myrcene. The concentration of this compound was ten times superior in the flower oils relative to the leaf oils. Higher levels of *p*-cymene were observed in the leaf oils than in the flower 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 the1,8-cineole type, nevertheless the scattering of this wild plant on irregular and stony fields, the increasing labor cost and the successive years of drought 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 way, the aerial parts of *T. mastichina* maintained in pots, on a sandy soil and fertilized every fifteen days with a solution 1:3:1 (N:P:K) and 0.4 % Mg, were collected over a day period, during the flowering phase. The oils were isolated from both the fresh leaves and the fresh flowers by hydrodistillation and analysed by GC and GC/MS.

MATERIAL AND METHODS

Plant Material

Each 5 cm long cutting obtained from aerial parts of fifteen plants of *T. mastichina* randomly collected at S. Brás de Alportel (Algarve), was placed in speedlings 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 under a temperature of 10-15 ºC and 50 % humidity and were sprayed every week (1 L/speedling). After 90 days, rooted cuttings were

transplanted to 2.5 L pots and placed under outside conditions. The plant material was maintained in pots (three plants each), on a sandy soil and fertilized every fifteenth day with a solution 1:3:1 (N:P:K) and 0.4 % Mg.

Isolation Procedure

The oils were isolated from fresh plant material 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, 70 eV; 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 essential oil yields isolated from *T. mastichina* are depicted 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. More significant differences were detected in the yields of leaf oils than in the flower ones, since a double oil amount was obtained in the evening (0.8 % and 0.9 %, at 23 and 17 h, respectively), compared to that obtained in the morning (0.4 % either at 8 and 12 h).

The percentages of the main components isolated from the oils of *T. mastichina* maintained in pots are given in Table 2. In quantitative terms, the most important component in the essential oils was 1,8-cineole, the concentrations of which were always highest in the leaf oils. The highest percentages registered in the leaf and 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. However, the most significant quantitative differences were detected for camphor. The percentages of this compound detected in the leaf oils (7.6 - 10.2 %) were almost double those observed in the flower oils (4.4 - 4.6 %). 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 %, respectively). In contrast, higher levels of linalool and α-terpineol were always present in the flower oils than in the leaf ones. Therefore, 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 or flower oils. The flower oils were always richest in α-pinene, sabinene, β-pinene and myrcene, the most important difference being observed for myrcene. In this case, six to ten times more myrcene were

registered in the flower oils than in the leaf oils, that is, 1.5 % to 1.6 % were detected in the flower oils, whereas in the leaf oils such percentages 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 %). In the sesquiterpene group of compounds, either oxygenated or not, elemol was the only one whose concent,ration in practically all samples, was superior to 1.0 %, the flower oils being richest in this component (2.3 % was the most elevated percentage, detected at 17 h).

Quantitative differences were observed either in the oil yields or in some components of the essential oils when comparing the leaf and the flower oils. Nevertheless, less significant quantitative differences were detected in some components of the oils according to the period of the day in which the plant had been collected.

Literature Cited

Garcia Vallejo, M.C., Garcia Martin, D., Muñoz, F. and Bustamante, L. 1984. Avance de un estudio sobre las esencias de *Thymus mastichina* (L.) L. español ("mejorana de España"). *1as Jornadas Nacionais de Plantas Aromáticas e Óleos Essenciais*, Coimbra, Portugal.

Tables

Table 1. Essential oil yields isolated from the leaves and the flowers of *T. mastichina* (L.) L. subsp. *mastichina* (percentage, v/w)

Part of plant	Time (h)			
	8:00	12:00	17:00	23:00
Leaf	0.4	0.4	0.9	0.8
Flower	1.7	2.0	2.2	1.6

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

		Lf.	Fl.	Lf.	Fl.	Lf.	Fl.	Lf.	Fl.
Component	Time (h)								
	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.