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Study of the composition of the different parts of a Spanish *Thymus vulgaris* L. plant

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The yield and composition of the dichloromethane extracts of leaves, flowers and stems of *Thymus vulgaris* L. growing in northeastern Spain have been studied. The yield obtained from leaves and flowers is much higher than that obtained from stems. This plant has a chemotype different from those previously known for *Thymus vulgaris* L. The fraction of the dichloromethane extract from leaves and flowers, studied by gas chromatography/mass spectrometry, shows that these tissues have higher concentrations of terpene hydrocarbons, oxygen terpene derivatives, sesquiterpene hydrocarbons, oxygen sesquiterpene derivatives, saturated aliphatic hydrocarbons and phytosterol derivatives than the stems. However, aldehydes with large number of carbon atoms have been foun ' in higher concentrations in stems than in flowers and leaves. These extracts from different parts of *Thymus vulgaris* L. also show the presence of a large number of flavonoids and vitamin E, compounds of great interest in food industry for their antioxidant activity. Leaves and flowers of this plant are of interest as flavourings, as well as being natural antioxidants for the food industry. © 1998 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

The renewed interest in natural products, rather than in synthetic agents, has again focused attention on plants as a source of flavourings (Yaylayan, 1991). In fact the number of spices used in food processing has been high since antiquity (Tainter and Grenis, 1993). However, in some cases, the same spice name is applied, in a generic way, to the different vegetable species of the same plant, without taking into account that even the same vegetable species can have several chemotypes, each one with a specific composition.

The genus *Thymus* has numerous species and varieties (Pignatti, 1982; García, 1985; Font, 1990; Rivera and Obón de Castro, 1991; De Bolòs *et al.*, 1993), and the composition of the essential oil of many of the *Thymus* species has been studied (Falchi, 1967; Miquel *et al.*, 1976; Passet, 1979; Bellomaría *et al.*, 1981; Adzet *et al.*, 1988*a*, 1989*a,b*, 1991; Ribeiro Salgueiro, 1992; Biondi *et al.*, 1993; Figuerido *et al.*, 1993; Panizzi *et al.*, 1993; Bellomaría *et al.*, 1994; Salgueiro *et al.*, 1995; Saez, 1995*a,b*; Vila *et al.*, 1995; Husnu Can Baser *et al.*, 1996; Senatore, 1996). Thyme has always been considered as an spice obtained from *Thymus vulgaris* L. (Tainter and Grenis, 1993) and it is used in savoury formulations, sauces, liqueurs, etc.

However, from *T. vulgaris* L. growing in France and in other countries (Granger and Passet, 1973; Piccaglia and Marotti, 1993) six chemotypes have been described whose principal components are geraniol, linalool, α-terpineol, carvacrol, thymol and *trans*-thujan-4-ol/terpinen-4-ol; from *T. vulgaris* L., growing in Spain, another chemotype with 1,8-cineole as its main component has been described (Adzet *et al.*, 1977). The composition of the spice thyme can be very different from one location to another and the essential oil of *T. vulgaris* L. can correspond to different blends of components with very different activities and organoleptic properties.

For this reason, it is very important to determine the real composition of the wild plants, growing in several regions, in the broadest and most accurate way. In this paper the composition of the dichloromethane extracts of stems, leaves and flowers of *T. vulgaris* L., growing wild in northeastern Spain is studied by gas chromatography and gas chromatography/mass spectrometry. Though essential oils are generally obtained from the entire aerial parts of the plant, this study will allow us to know the differences between the composition of the three parts of the herb, and also to what extent these three parts can afford flavourings and extracts with different activities and organoleptic properties.

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MATERIALS AND METHODS

Samples and extraction

The samples were collected in the spring of 1993 in Zaragoza (Aragón, Spain). They were air-dried and ground in a refrigerated mill (Janke and Kunkel IKA-Labortechnik A10), to avoid loss of the most volatile components. Dichloromethane extracts were obtained using an ultrasonic bath for 1 h in order to ensure an exhaustive extraction. Replicate extraction experiments were carried out.

The study of the composition of the extracts was carried out by means of gas chromatography (GC) and gas chromatography/mass spectrometry (GC–MS) techniques. Replicate analyses were performed.

Gas chromatography

A Hewlett-Packard gas chromatograph model 5890 series II, equipped with a Flame Ionization Detector (FID) and a Hewlett-Packard Vectra VL2 4/66 computer, were used for the quantitative study. A fused-silica capillary column (30 m long, 0.32 mm i.d. and 0.25 μm film thickness), coated with a non-polar stationary phase (Hewlett-Packard 5 cross-linked 5% phenyl methyl silicone) was used. The temperature programme began at 50°C (0.5 min) with an increase of 5°C/min until 290°C (10 min). Nitrogen was used as carrier gas. The injection technique used was split with a split ratio 1:10. Injections of 1 μl were made. Injector and detector temperatures were 250 and 300°C, respectively. α-Terpinene, camphor, *trans*-caryophyllene, nerolidol, pentacosane, 3-methyl-2 (5H)-furanone, phytol, squalene, vitamin E, β-sitosterol and flavanone were used as external standards for quantification. These compounds are available from Aldrich, Fluka and Sigma.

GC–MS identification

A Hewlett-Packard 5973 mass selective detector and interfaced 6890 model series gas chromatograph, and a Hewlett-Packard Vectra pentium computer were used. A fused-silica capillary column (30 m long, 0.25 mm i.d. and 0.25 μm film thickness), coated with a non-polar stationary phase (Hewlett-Packard 5 cross-linked 5% phenyl methyl silicone) was used. The temperature programme began at 50°C (0.5 min) with an increase of 5°C/min until 300°C (10 min). Helium was used as carrier gas. Injector and detector temperatures were 250 and 280°C, respectively. The injection technique used was split with a split ratio 1:10 and injections of 1 μl were made. The mass spectra were recorded at an ionization energy of 70 eV. The volatile components were identified, as in previous studies (Blanco *et al.*, 1991; Guillén *et al.*, 1992; Guillén and Manzanos, 1994, 1996, 1997), by comparing of retention times with those of pure substances, by co-elution with standards, and by mass spectrometry. The pure substances used for identification were available from Aldrich, Fluka and Sigma, and are asterisked in Table 1. For those peaks, for which the corresponding pure compound was not available, identification was performed by matching their mass spectra to those of the Wiley mass spectra library (Wiley, 1990).

RESULTS AND DISCUSSION

The average relative weights of leaves, L, flowers, F, and stems, S, in the *T. vulgaris* L. plant here studied were 34.2%, 9.9% and 55.8%, respectively. The extraction yield obtained from leaves, flowers and stems was 4.0%, 2.6% and 0.5%, respectively, showing that leaves and flowers are the parts of the plant that contain the highest proportion of compounds extractable with dichloromethane. It must be pointed out that dichloromethane extracts are constituted by compounds both detectable and not detectable by gas chromatography.

Figure 1 shows the total ion chromatograms obtained from these extracts; it is evident that the composition of these samples is different and so also are their activities and organoleptic properties. The total number of identified compounds was 171 and these, together with their concentrations reported in mg/kg in the plant tissues, assuming that dichloromethane is able to extract the total amount of each compound present in the corresponding tissue, are given in Tables 1 and 2. The number of unidentified compounds was 57 and the main mass fragments of their mass spectra are also given in Tables 1 and 2, together with their concentration in the corresponding plant tissue; for the majority of the unidentified compounds, their nature has been predicted as a function of their mass spectra, although their specific assignation to concrete compounds has not been possible with the available data.

Terpene hydrocarbons are found in small concentrations in the three tissues (L = 448.9 mg/kg, F = 162.1 mg/kg and S = 0.7 mg/kg), especially in stems. The same terpene hydrocarbons, except tricyclene, were detected in leaves and flowers, but in different concentrations; α-pinene, camphene and β-pinene are the main terpene hydrocarbons in leaves.

Oxygen terpene derivatives constitute the main concentration in leaves and flowers and a small concentration in stems (L = 2254.6 mg/kg, F = 1079.3 mg/kg and S = 25.9 mg/kg). Leaf extract is richest in oxygen terpene derivatives; a total of 50 was detected, in contrast to 38 and 43 in flowers and stems, respectively. The main oxygen terpene derivatives in the three extracts are 1,8-cineole, linalool, followed by camphor, endo-borneol, α-terpineol and linalyl acetate; however, it is noteworthy that the main oxygen derivative in leaves is 1,8-cineole and, in flowers, is linalool. From these results it is deduced that the *T. vulgaris* L. studied here has a chemotype different (1,8-cineole and linalool) from that previously studied in plants from Southern Europe and

Table 1. Identified and unidentified components in the leaves (L), flowers (F) and stems (S) of *Thymus vulgaris* L. dichloromethane extracts, together with their concentrations in mg/kg in each plant tissue

No.	Components	L	F	S
Terpene hydrocarbons		449	162	0.7
1	Tricyclene[a]	8.1	—	—
2	α-Thujene	7.2	2.7	tr
3	α-Pinene[a]	86.3	23.7	tr
4	Camphene[a]	86.4	20.3	0.3
5	Sabinene	33.4	22.5	tr
6	β-Pinene[a]	113.1	40.1	0.4
7	β-Myrcene[a]	34.3	23.4	tr
8	*p*-Cymene[a]	23.7	2.1	tr
9	Limonene[a]	nd	nd	nd
10	*cis*-Ocimene	6.6	2.2	—
11	*trans*-Ocimene	36.0	20.1	—
12	γ-Terpinene[a]	13.8	5.0	tr
13	Alloocimene	tr	tr	—
Oxygen terpene derivatives		2255	1079	25.9
14	1,7-exo-Trimethylenebicyclo[3.2.1]octane	2.3	tr	tr
15	1,8-Cineole[a]	947	254	13.9
16	*trans*-Sabinene hydrate	27.2	21.1	0.3
17	*cis*-Linalool oxide	20.1	4.4	0.2
18	*trans*-Linalool oxide	19.8	4.4	0.3
19	Linalool[a]	441	525	1.8
20	*cis*-*p*-Menth-2-en-1-ol	5.7	—	tr
21	α-Campholene aldehyde	5.8	—	tr
22	*trans*-Pinocarveol	14.6	—	0.2
23	Camphor[a]	107	33.9	1.5
24	5-(1-Methylethyl)-bicyclo[3.1.0]hexan-2-one (sabina ketone)	5.9	—	tr
25	3,5-Dimethyl-4-ethylidenecyclohex-2-ene-1-one	3.7	—	tr
26	endo-Borneol[a]	167	34.3	2.9
27	Epoxylinalool	nd	nd	nd
28	Epoxylinalool (isomer)	7.4	1.3	tr
29	Terpinen-4-ol[a]	23.3	3.9	0.3
30	5-One-1,8-cineole	5.7	tr	tr
31	Cuminyl alcohol	4.2	—	tr
32	α-Terpineol[a]	184	64.0	2.6
33	Myrtenol	16.4	—	0.6
34	Unidentified (57(100), 69, 99, 113, 128, 152)	—	4.0	—
35	Isopulegone	16.8	3.2	0.3
36	Unidentified (71, 83, 108(100), 111, 126, 170)	nd	nd	nd
37	*trans*-Carveol	4.8	tr	tr
38	Unidentified (71, 83, 108(100), 126, 170)	12.5	1.1	0.3
39	β-Citronellol	10.1	1.9	—
40	5-Hydroxy-1,8-cineole	10.5	tr	0.2
41	Linalyl acetate[a]	77.6	66.4	tr
42	Neryl formate	6.8	2.7	tr
43	endo-Bornyl acetate	22.8	7.3	0.3
44	Thymol[a]	tr	—	—
45	Geranyl formate	—	tr	—
46	Carvacrol[a]	tr	tr	tr
47	Campholytic acid methyl ester	tr	—	—
48	*cis*-*p*-Menth-2-ene-1,8-diol	tr	—	—
49	*trans*-*p*-Menth-2-ene-1,8-diol	11.6	—	tr
50	Citronellyl acetate	—	tr	—
51	Neryl acetate[a]	—	tr	—
52	Eugenol[a]	tr	—	—
53	endo-Bornyl propanoate	15.5	3.1	0.2
54	Geranyl acetate[a]	—	tr	—
55	*trans*-Sobrerol	7.9	tr	tr
56	4-Trimethyl-5-hydroxy-3-cyclohexene-1-methanol	5.2	—	tr
57	Unidentified (43, 55, 67, 69, 71(100), 82, 135, 153, 168)	—	39.4	—
58	*cis*-*p*-Menthenediol (isomer)	9.2	—	tr
59	Vanillin[a]	—	—	tr

(continued)

Table 1—*contd*

No.	Components	L	F	S
60	1,2-Dimethoxy-4-(2-propenyl)-benzene[a]	—	tr	—
61	endo-Bornyl isobutanoate	tr	—	tr
62	8-Hydroxycarvotanacetone	tr	—	tr
63	Citronellyl propanoate	tr	tr	—
64	Neryl propanoate	nd	tr	—
65	endo-Bornyl butanoate (isomer)	7.1	—	tr
66	Geranyl propanoate	5.0	3.0	tr
67	*p*-Menthane-1,2,4-triol	tr	—	—
68	*p*-Menthanetriol	17.3	—	tr
69	Neryl isobutanoate	tr	tr	tr
70	Geranyl isobutanoate	6.4	1.0	tr
71	Geranyl butanoate (isomer)	tr	tr	tr
Sesquiterpene hydrocarbons		88.9	73.7	0.4
72	β-Bourbonene	10.2	1.8	tr
73	Isocaryophyllene	—	tr	—
74	α-Gurjunene	tr	—	—
75	*trans*-Caryophyllene[a]	35.3	46.6	0.2
76	Calarene	8.9	tr	tr
77	Unidentified (57, 79, 93, 109, 137, 152, 161(100), 204)	—	1.4	—
78	α-Bergamotene	—	—	0.2
79	Aromadendrene	—	tr	—
80	α-Humulene	4.2	2.2	—
81	Alloaromadendrene	tr	3.2	tr
82	Unidentified (105, 119, 133, 161(100), 189, 204)	tr	tr	—
83	1,2,3,4,4a,5,6,8a-Octahydro-7-methyl-4-methylene-1-(1-methylethyl)-napthalene	18.3	5.8	tr
84	Farnesene	—	tr	—
85	Germacrene B	tr	3.9	—
86	γ-Cadinene	3.6	1.1	tr
87	Calamenene	5.7	2.9	tr
88	Unidentified (43, 55, 67, 81, 91(100), 105, 119, 131, 159, 177, 187, 202)	—	2.9	—
89	Unidentified (81, 93, 105, 119, 161(100), 179, 204)	2.7	1.9	tr
Oxygen sesquiterpene derivatives		537	182	6.2
90	Hedicaryol	—	tr	—
91	Elemol	163	56.8	0.9
92	Aristolone	tr	—	—
93	Unidentified (81(100), 91, 105, 123, 134, 161, 207, 222)	nd	4.1	—
94	Spathulenol	24.7	4.3	0.3
95	Caryophyllenol II	49.5	11.3	1.0
96	Viridiflorol	12.8	—	0.6
97	Calarene epoxide	tr	tr	tr
98	Unidentified (91, 105, 133, 161, 189(100), 204, 222)	5.7	tr	tr
99	Unidentified (43, 55, 69, 81, 93, 111(100), 137, 153, 168, 180, 220)	13.0	—	tr
100	Unidentified (79, 91, 105, 161, 189(100), 204, 222)	14.4	3.3	tr
101	Unidentified (69, 79, 91, 109, 136(100), 177, 218)	18.7	3.7	0.3
102	Aristolene epoxide	11.5	25.3	tr
103	β-Eudesmol	37.7	10.5	0.7
104	Torreyol	32.3	12.4	0.5
105	Unidentified (55, 67, 82, 93, 111(100), 123, 220)	7.6	tr	tr
106	Unidentified (79, 91(100), 107, 121, 131, 149, 159, 187, 220)	16.8	5.2	tr
107	Unidentified (43, 55, 67, 81, 93, 111(100), 197, 212, 202, 220)	6.5	tr	tr
108	Unidentified (79, 91, 105, 119, 131, 159(100), 177, 202, 220)	—	9.3	—
109	Unidentified (43, 55, 69, 81, 91, 109(100), 123, 137, 159, 182, 202, 220)	12.0	—	tr
110	Unidentified (43, 55, 67, 81, 93, 111(100), 236)	9.9	—	—
111	Unidentified (79, 84(100), 93, 107, 121, 137, 159, 187, 202, 220, 238)	25.6	11.5	—
112	7-Acetyl-2-hydroxy-2-methyl-5-isopropylbicyclo[4.3.0]nonane	tr	tr	tr
113	Unidentified (55, 69, 79(100), 93, 107, 125, 136, 149, 203, 218)	—	3.7	—
114	Unidentified (43, 55, 67, 70, 79(100), 93, 107, 123, 149, 162, 175, 234)	17.4	—	0.5
115	Unidentified (59, 67, 81(100), 93, 109, 122, 135, 175, 203, 218)	—	4.4	—
116	Unidentified (55, 69, 81, 95, 109, 123(100), 127, 161, 179, 202, 220)	7.4	—	tr
117	Unidentified (55, 67, 79, 91, 109, 135, 149(100), 160, 175, 203, 221, 236)	33.7	6.7	0.8
118	Unidentified (59, 80, 91, 107, 120, 134, 147(100), 162, 177, 205, 236)	6.1	tr	tr
119	Unidentified (55, 68, 82, 95(100), 109, 123, 205, 220)	tr	3.6	0.6
120	Unidentified (57, 67, 77, 91, 105(100), 133, 147, 159, 175, 187, 205, 220)	—	3.8	—
121	Unidentified (133, 145, 159(100), 187, 202, 220)	11.2	2.4	—

Table 1—*contd*

Acids		tr	tr	tr
122	Tetradecanoic acid	—	—	tr
123	Hexadecanoic acid[a]	tr	tr	tr
124	9,12-Octadecadienoic acid	tr	tr	tr
125	9-Octadecenoic acid[a]	tr	tr	tr
126	Octadecanoic acid	tr	tr	tr
127	Eicosanoic acid	—	—	tr
Aldehydes		tr	—	26.0
128	Eicosanal	—	—	1.0
129	Docosanal	—	—	0.5
130	Tricosanal	—	—	tr
131	Tetracosanal	—	—	5.0
132	Pentacosanal	—	—	0.4
133	Hexacosanal	tr	—	13.0
134	Heptacosanal	—	—	0.2
135	Octacosanal	tr	—	4.1
136	Triacontanal	tr	—	1.8
137	Tetratriacontanal	tr	—	—
Saturated hydrocarbons		739	320	61.4
138	Octadecane[a]	—	—	tr
139	Nonadecane[a]	—	—	tr
140	Eicosane[a]	—	—	tr
141	Heneicosane[a]	—	—	tr
142	Docosane[a]	—	tr	tr
143	Tricosane[a]	—	tr	0.2
144	Methyl docosane	—	tr	—
145	Tetracosane[a]	—	0.9	tr
146	Ethyl docosane	—	2.8	—
147	Methyl tricosane	—	tr	—
148	Pentacosane[a]	tr	1.7	0.3
149	Ethyl tricosane	—	tr	—
150	Methyl tetracosane	tr	6.8	tr
151	Hexacosane[a]	tr	tr	tr
152	Ethyl tetracosane	tr	4.0	—
153	Methyl pentacosane	—	1.6	—
154	Heptacosane[a]	4.9	7.4	0.5
155	Methyl hexacosane	tr	11.2	tr
156	Octacosane[a]	14.9	tr	0.4
157	Ethyl hexacosane	2.6	10.9	0.5
158	Methyl heptacosane	tr	6.6	0.4
159	Nonacosane[a]	105	58.0	10.4
160	Ethyl heptacosane	tr	2.3	tr
161	Methyl octacosane	5.4	22.3	0.6
162	Triacontane[a]	22.5	7.4	1.2
163	Ethyl octacosane	9.6	10.9	0.7
164	Methyl nonacosane	6.5	9.1	—
165	Hentriacontane	165	54.2	10.0
166	Ethyl nonacosane	4.1	2.1	0.5
167	Methyl triacontane	14.2	14.4	1.6
168	Dotriacontane	29.5	5.8	1.9
169	Ethyl triacontane	12.2	5.5	1.2
170	Methyl hentriacontane	11.5	9.9	14.3
171	Tritriacontane	214	33.7	13.7
172	Ethyl tritriacontane	20.9	—	—
173	Methyl dotriacontane	37.2	17.8	nd
174	Tetratriacontane	25.5	12.5	1.6
175	Ethyl dotriacontane	8.8	—	—
176	Methyl tritriacontane	tr	tr	tr
177	Pentatriacontane	25.9	tr	1.4
178	Methyl tetratriacontane	tr	—	—
179	Methyl pentatriacontane	tr	—	—
Phytosterols and other derivatives		201	157.0	80.4
180	5-Methyl-5-ethenyldihydro-2(3H)-furanone	11.0	—	tr
181	Hexyl butanoate[a]	nd	nd	nd

(continued)

Table 1—*contd*

No.	Components	L	F	S
182	Unidentified (43, 57, 74, 85, 98, 103(100), 111, 158)	tr	4.0	—
183	Unidentified (43, 55, 67(100), 71, 82)	54.6	11.2	0.9
184	Unidentified (43, 55, 67, 71(100), 82)	12.4	tr	tr
185	3-(1-Methyl-2-pyrrolidinyl)-pyridine (nicotine)	—	—	tr
186	Unidentified (43, 55, 67, 71, 82(100))	5.4	47.4	tr
187	5,6,7,7a-Tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone (dihydroactinidiolide)	5.2	tr	0.3
188	5,6,7,7a-Tetrahydro-6-hydroxy-4,4,7a-trimethyl-2(4H)-benzofuranone (loliolide)	—	tr	—
189	Neophytadiene[a]	6.7	—	—
190	6,10,14-Trimethyl-2-pentadecanone (1,2-dinor-3-phytanone)	tr	1.8	0.3
191	Unidentified (196, 213, 242(100))	—	—	0.3
192	Unidentified (57, 69, 88, 97, 111, 127, 145(100), 196, 224, 269, 368)	—	—	0.3
193	Squalene[a]	7.8	tr	0.2
194	Unidentified (135, 143, 275, 394(100))	tr	—	1.4
195	Vitamin E[a]	4.4	nd	0.9
196	Ergost-5,24-dien-3-β-ol	—	tr	—
197	(24R)-Ergost-5-en-3β-ol (campesterol)[a]	tr	3.8	tr
198	Phytosterol derivative (201, 255, 271, 300, 412, 444(100))	—	tr	—
199	Stigmast-5-en-3β-ol (β-sitosterol)[a]	54.8	37.5	13.2
200	Unidentified (175, 189, 204(100), 412)	29.3	18.0	6.1
201	β-Amyrin	—	12.8	7.9
202	Unidentified (134, 147, 161, 175, 184, 204(100), 412)	nd	—	7.7
203	Unidentified (163(100), 190, 396, 412)	tr	—	3.1
204	α-Amyrin (viminalol)	tr	nd	5.3
205	Stigmasta-3,5-dien-7-one	tr	—	6.7
206	Unidentified (175, 189, 204(100), 382, 446)	—	—	3.1
207	Stigmast-4-en-3-one (β-sitostenone)	—	18.5	7.7
208	Unidentified (57, 68, 82, 95, 123(100), 278, 296, 534)	9.2	tr	—
209	Unidentified (175, 189, 203(100), 232)	—	—	2.4
210	Unidentified (245, 287, 316, 428(100), 647, 662)	tr	2.0	2.5
211	Unidentified (137, 150, 177, 194(100), 474)	—	—	1.0
212	Unidentified (133, 175, 190, 203(100), 446)	—	tr	1.4
213	Unidentified (175, 189, 203(100), 232, 249, 446)	—	—	3.0
214	Unidentified (137, 150, 177, 194(100), 474)	—	—	4.7

[a]Pure substances used as standars for identification; tr, traces; nd, not determined.

it could be considered as an intermediate between the linalool chemotype found in France and the 1,8-cineole chemotype found in Spain for *T. vulgaris* L. (Granger and Passet, 1973; Adzet *et al.*, 1977).

Sesquiterpene hydrocarbons are found in small concentrations in the three tissues (L = 88.9 mg/kg, F = 73.7 mg/kg and S = 0.4 mg/kg). The number of sesquiterpene hydrocarbons detected is 12, 16 and 9 in leaves, flowers and stems, respectively, and the main component is *trans*-caryophyllene.

Oxygen sesquiterpene derivatives are present in the three tissues in small concentrations (L = 537.4 mg/kg, F = 182.3 mg/kg and S = 6.2 mg/kg). The number of unidentified compounds in this group is high; this fact is due to the small concentration of these compounds in the samples and to the complexity of their mass spectra. The number of detected compounds in this group varies from 27 in leaves to 25 in flowers and 22 in stems.

From the above it can be concluded that three different flavourings can be obtained from leaves, flowers and stems of *T. vulgaris* L. of the same plant.

In addition to the compounds responsible for the organoleptic properties above mentioned, other compounds have been found. Fatty acids, at trace levels, have been detected in the three extracts. Aldehydes with a large number of carbon atoms have been found in stems at a concentration of 26.0 mg/kg; some of these compounds have also been detected in leaves, at trace level. Saturated hydrocarbons with large number of carbon atoms have been found in the three tissues (L = 739.3 mg/kg, F = 320 mg/kg and S = 61.4 mg/kg).

Noteworthy is the presence of α-tocopherol and of a group of sterol derivatives [ergost-5,24-dien-3β-ol, (24R) -ergost-5-en-3β-ol (campesterol), stigmast-5-en-3β-ol (β-sitosterol), β-amyrin, α-amyrin (viminalol), stigmasta-3,5-dien -7-one, stigmast-4-en-3-one (β-sitostenone)] found in the three parts of the plant in small concentrations.

Finally Table 2 gives the flavonoids detected in the three samples. The highest concentration of these compounds has been found in flowers, however, the highest number of derivatives is in leaves. The main mass fragments of the mass spectra of the identified and of the unidentified flavonoids are given. All of them show the typical mass fragmentations of flavonoids (Markham, 1982). Of the 14 flavonoid derivatives found in leaves, only four compounds, two flavanones and two flavones have been identified (compound 3: 5,4′-dihydroxy-7-methoxyflavanone (sakuranetin or 7-methylnaringenin,

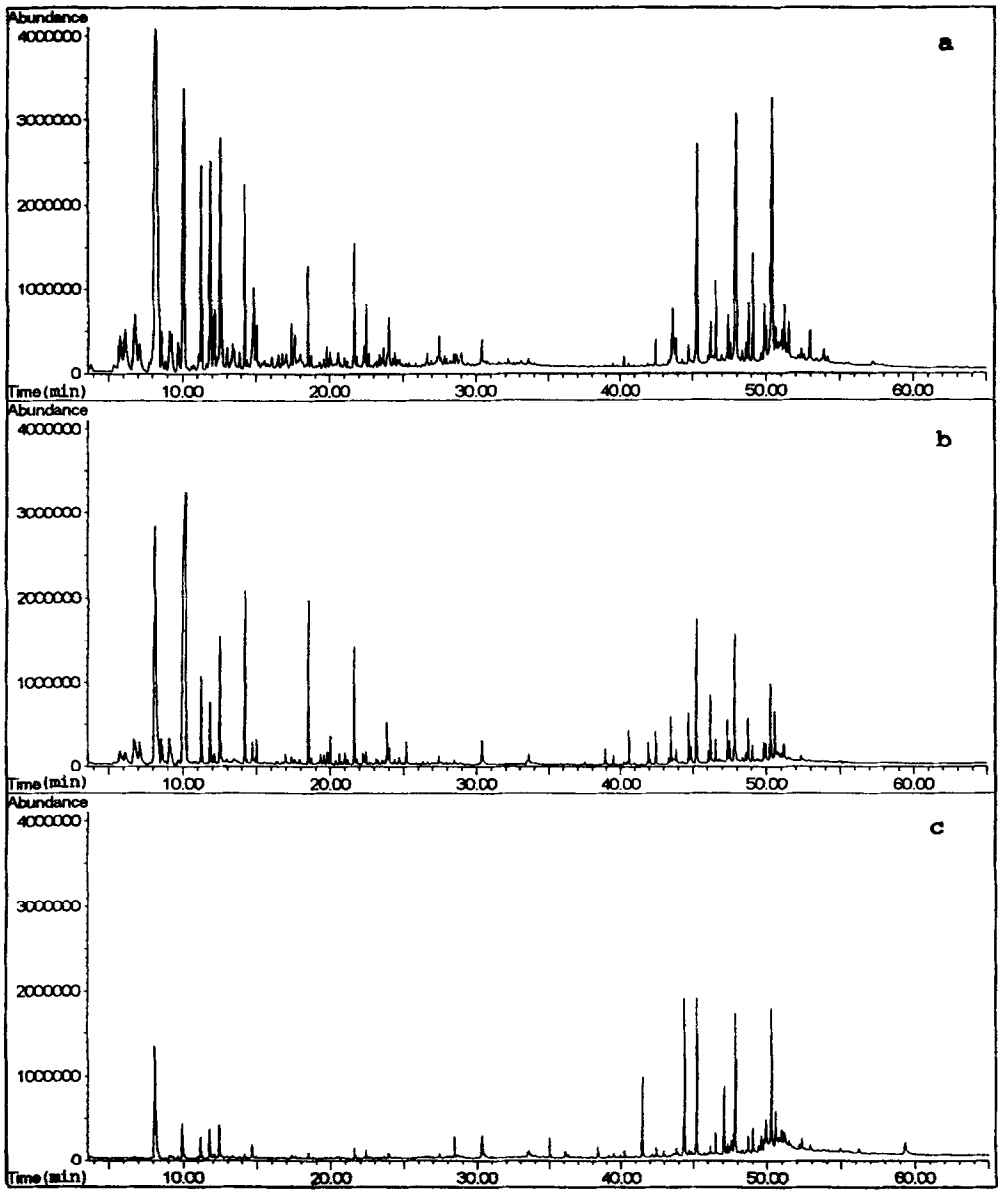

Fig. 1. Total ion chromatograms of (a) the leaves, (b) flowers and (c) stems of *Thymus vulgaris* L. dichloromethane extracts.

MW = 286); compound 7: 5,4′-dihydroxy-6,7-dimethoxyflavanone (6,7-dimethylcarthamidin, MW = 316); compound 13: 5-hydroxy-6,7,3′,4′-tetramethoxyflavone (5-desmethylsinensetin, MW = 358) and tentatively compound 14: 5-hydroxy-3,7,8,2′,4′-pentamethoxyflavone, MW = 388). The difficulty in identifying the detected flavonoids is due to the mass spectral data of many of these compounds not being available (Markham, 1982; Van den Broucke *et al.*, 1982; Ferreres *et al.*, 1985; Harborne, 1988, 1994; Miura and Nakatani, 1989). Compound numbers 3 and 13 have been found by Adzet *et al.* (1988*b*) and other authors (Ferreres *et al.*, 1985; Hernández *et al.*, 1987), respectively, in different *Thymus* subspecies.

The usual methodology for studying the flavonoid derivatives in plants involves successive extractions using more than one solvent, several fractionating steps and different chromatography techniques to extract, separate, isolate, purify and identify the compounds of interest. Table 3 lists the flavonoids found in *T. vulgaris* L. by several authors, using the methodology above mentioned, together with the main mass fragment of their mass spectra for those that are available.

It can be observed that Van den Broucke *et al.* (1982) have detected four flavonoids in *T. vulgaris* L.: 5,6,4′-trihydroxy-7,8,3′-trimethoxyflavone (thymonin, MW = 360) and 5,4′-dihydroxy-6,7,3′-trimethoxyflavone (cirsilineol, MW = 344), whose mass spectra fragments given by Van den Broucke *et al.* (1982) and Miura and Nakatani (1989) are not totally in agreement with those of the compound numbers 10, 11 and 12 in Table 2; and 5,7,4′-trihydroxyflavanone (naringenin, MW = 272) and 5,4′-dihydroxy-6,7,8,3′-tetramethoxyflavone (7-methylsudachitin, MW = 374) not detected here.

Table 2. Ion molecular and main fragments (*m/z* (%)) of the mass spectra of the identified and the unidentified flavonoids in the leaves (L), flowers (F) and stems (S) of *Thymus vulgaris* L. dichloromethane extracts, and their concentrations in mg/kg in each plant tissue

No.	Compound	L	F	S
1	Dihydroxy-methoxyflavanone or tetrahydroxyflavone (286 (M^+, 100), 272(6), 258(10), 243(8), 206(8), 193(39), 180(35), 167(90), 138(18), 120(36), 95(22))	tr	—	—
2	Dimethoxyflavanone or dihydroxy-methoxyflavone (284 (M^+, 83), 269(100), 251(36), 239(45), 211(12), 183(17), 171(28), 157(35), 141(13))	tr	1.9	tr
3	5,4′-Dihydroxy-7-methoxyflavanone (7-methylnaringenin or sakuranetin) (286 (M^+, 100), 269(8), 243(4), 193(31), 180(32), 167(87), 138(17), 120(28), 95(15))	tr	8.1	tr
4	Dihydroxy-dimethoxyflavanone or tetrahydroxy-methoxyflavone (316 (M^+, 100), 300(15), 284(38), 230(9), 193(28), 180(64), 167(92), 150(55), 137(64))	tr	—	—
5	Dihydroxy-dimethoxyflavanone or tetrahydroxy-methoxyflavone (316 (M^+, 100), 301(8), 267(10), 230(3), 181(30), 135(12), 120(17))	tr	—	—
6	Dihydroxy-trimethoxyflavanone or tetrahydroxy-dimethoxyflavone (346 (M^+, 100), 315(10), 286(6), 196(70), 181(66), 167(19), 153(23), 136(8), 123(9))	tr	—	—
7	5,4′-Dihydroxy-6,7-dimethoxyflavanone (6,7-dimethylcarthamidin) (316 (M^+, 99), 301(9), 286(2), 257(6), 223(5), 196(74), 181(100), 168(10), 153(21), 136(4), 120(10))	tr	—	—
8	Dihydroxy-trimethoxyflavanone or tetrahydroxy-dimethoxyflavone (346 (M^+, 78), 331(5), 316(54), 301(6), 226(90), 211(100), 196(49), 181(60), 167(17), 153(23), 135(18))	tr	tr	—
9	Dihydroxy-tetramethoxyflavanone or tetrahydroxy-trimethoxyflavone (376 (M^+, 15), 346(100), 331(8), 260(1), 226(22), 211(19), 196(68), 181(78), 168(12), 150(19), 135(14))	tr	tr	—
10	Hydroxy-tetramethoxyflavanone or trihydroxy-trimethoxyflavone (360 (M^+, 100), 345(7), 196(80), 181(65), 164(59), 151(19))	tr	—	—
11	Tetramethoxyflavanone or dihydroxy-trimethoxyflavone (344 (M^+, 59), 329(100), 314(35), 299(38), 267(24), 253(27), 239(19), 183(27), 153(30), 135(43))	tr	—	—
12	Tetramethoxyflavanone or dihydroxy-trimethoxyflavone (344 (M^+, 100), 329(71), 315(30), 298(31), 283(8), 264(13))	tr	tr	—
13	5-Hydroxy-6,7,3′,4′-tetramethoxyflavone (5-desmethylsinensetin) (358 (M^+, 100), 343(93), 329(18), 315(22), 312(26), 299(6), 282(5))	tr	tr	—
14	5-Hydroxy-3,7,8,2′4′-pentamethoxyflavone (388 (M^+, 61), 373(100), 355(5), 299(22), 211(13), 155(12))	tr	tr	—

tr, traces.

In addition to the compounds detected by the authors above mentioned, eight other flavonoids have been detected by Hernández *et al.* (1987) in *T. vulgaris* L., namely: 5,4′-dihydroxy-6,7-dimethoxyflavone (cirsimaritin, MW = 314), 5-hydroxy-6,7,4′-trimethoxyflavone (salvigenin, MW = 328), 5,6,4′-trihydroxy-7,8-dimethoxyflavone (thymusin, MW = 330), 5-hydroxy-6,7,8,4′-tetramethoxyflavone (gardenin-B, MW = 358) and 5,3′,4′-trihydroxy-6,7,8-trimethoxyflavone (sideritoflavone, MW = 360) not found in the plant studied here; 5-hydroxy-6,7,3′,4′-tetramethoxyflavone (5-desmethylsinensetin, MW = 358) also detected in this study; 5-hydroxy-6,7,8,3′,4′-pentamethoxyflavone (5-desmethylnobiletin, MW = 388) that could be the unidentified compound number 14 in Table 2; and finally 5,4′-dihydroxy-6,7,8-trimethoxyflavone (xanthomicrol, MW = 344), whose mass spectra fragments are not in total agreement with compound numbers 11 and 12 in Table 2.

Adzet *et al.* (1988*b*) have detected, in *T. vulgaris* L., some other flavonoids, such as 5,7,4′-trihydroxyflavone (apigenin, MW = 270), 5,7,3′,4′-tetrahydroxyflavanone (eriodictyol, MW = 288), 3,5,7,4′-tetrahydroxyflavanone (dihydrokaempferol or aromadendrin, MW = 288), 3,5,7,4′,5′-pentahydroxyflavanone (dihydroquercetin or taxifolin, MW = 304) and 5,4′-dihydroxy-6,7,8-trimethoxyflavanone (dihydroxanthomicrol, MW = 346) not found in this study. These authors also identified 5,4′-dihydroxy-7-methoxyflavone (genkwanin or 7-methylapigenin, MW = 284), whose mass fragments do not agree with those of compound number 2 in Table 2 and 5,7,3′,4′-tetrahydroxyflavone (luteolin, MW = 286) that could be the unidentified compound number 1 in Table 2, and finally 5,4′-dihydroxy-7-methoxyflavanone (7-methylnaringenin or sakuranetin, MW = 286) detected in the plant studied here.

Miura and Nakatani (1989), using acetone as the solvent after removing the non-polar components of the leaves of *T. vulgaris* L., and following a complex fractionating scheme, found six flavones, one of them not detected before by the authors mentioned above, namely: 5-hydroxy-7,4′-dimethoxyflavone (7,4-dimethylapigenin, MW = 298) not found here.

Finally, Morimitsu *et al.* (1995) and Picuric-Jovanovic *et al.* (1995) detected, in *T. vulgaris* L., a flavonoid not detected before by the above-mentioned authors, namely: 3,5,7,3′,4′-pentahydroxyflavone (quercetin, MW = 302) not found in this study.

Differences between the composition in flavonoids in *T. vulgaris* L. from different origins can be due both to the several chemotypes existing in this plant species and to the different effectiveness and polarity of the solvents used to extract them.

From the results obtained it is evident that this *T. vulgaris* L. plant is a rich source not only of flavourings for

Table 3. Flavonoids found by several authors in *Thymus vulgaris* L., and the main mass fragments of the mass spectra of some of them

Flavonoids	References
5,7,4′-Trihydroxyflavanone (naringenin, MW = 272) (272 (M^+, 79), 253(6), 229(3), 179(31), 166(42), 153(100), 120(56))[a]	Van den Broucke *et al.*, 1982 Adzet *et al.*, 1988*b*
5,4′-Dihydroxy-6,7,3′-trimethoxyflavone (cirsilineol, MW = 344) (344 (M^+, 98), 343(21), 329(100), 315(22), 301(28), 181(21), 153(42), 151(9), 148(3))[b] (344 (M^+, 100), 329, 315, 314, 181, 153, 151, 149)[c]	Van den Broucke *et al.*, 1982 Hernández *et al.*, 1987 Adzet *et al.*, 1988*b* Miura and Nakatani, 1989 Morimitsu *et al.*, 1995
5,6,4′-Trihydroxy-7,8,3′-trimethoxyflavone (thymonin, MW = 360) (360 (M^+, 68), 359 (5), 345(100), 197(15), 169(4), 151(5), 148(< 2))[b]	Van den Broucke *et al.*, 1982 Hernández *et al.*, 1987 Morimitsu *et al.*, 1995
5,4′-Dihydroxy-6,7,8,3′-tetramethoxyflavone (7-methylsudachitin, MW = 374) (374 (M^+, 77), 373(2), 359(100), 211(17), 183(10), 151(4), 148(2))[b] (374 (M^+, 66), 359(100), 211(13), 183(12), 151(4), 148(3))[c]	Van den Broucke *et al.*, 1982 Hernández *et al.*, 1987 Adzet *et al.*, 1988*b* Miura and Nakatani, 1989
5,4′-Dihydroxy-6,7-dimethoxyflavone (cirsimaritin, MW = 314) (314 (M^+, 100), 299(70), 284(11), 271(51), 181(30), 121(19))[c]	Hernández *et al.*, 1987 Adzet *et al.*, 1988*b* Miura and Nakatani, 1989
5-Hydroxy-6,7,4′-trimethoxyflavone (salvigenin, MW = 328)	Hernández *et al.*, 1987
5,6,4′-Trihydroxy-7,8-dimethoxyflavone (thymusin, MW = 330)	Hernández *et al.*, 1987
5,4′-Dihydroxy-6,7,8-trimethoxyflavone (xanthomicrol, MW = 344) (344 (M^+, 63), 329(100), 211(15), 183(17), 118(11))[c]	Hernández *et al.*, 1987 Adzet *et al.*, 1988*b* Miura and Nakatani, 1989
5-Hydroxy-6,7,3′,4′-tetramethoxyflavone (5-desmethylsinensetin, MW = 358) (358 (M^+, 100), 343(82), 329(24), 315(25), 312(26), 163(19), 153(40))[a]	Hernández *et al.*, 1987
5-Hydroxy-6,7,8,4′-tetramethoxyflavone (gardenin-B, MW = 358)	Hernández *et al.*, 1987
5,3′,4′-Trihydroxy-6,7,8-trimethoxyflavone (sideritoflavone, MW = 360)	Hernández *et al.*, 1987 Adzet *et al.*, 1988*b*
5-Hydroxy-6,7,8,3′,4′-pentamethoxyflavone (5-desmethylnobiletin, MW = 388)	Hernández *et al.*, 1987 Adzet *et al.*, 1988*b*
5,7,4′-trihydroxyflavone (apigenin, MW = 270)	Adzet *et al.*, 1988*b*
5,4′-Dihydroxy-7-methoxyflavone (genkwanin or 7-methylapigenin, MW = 284) (284 (M^+, 100), 255(36), 241(16), 167(13), 166(14), 138(14), 128(16))[c]	Adzet *et al.*, 1988*b* Miura and Nakatani, 1989
5,4′-dihydroxy-7-methoxyflavanone (sakuranetin or 7-methylnaringenin, MW = 286)	Adzet *et al.*, 1988*b*
5,7,3′,4′-Tetrahydroxyflavone (luteolin, MW = 286)	Adzet *et al.*, 1988*b* Samejima *et al.*, 1995
5,7,3′,4′-Tetrahydroxyflavanone (eriodictyol, MW = 288)	Adzet *et al.*, 1988*b* Morimitsu *et al.*, 1995 Haraguchi *et al.*, 1996
3,5,7,4′-tetrahydroxyflavanone (dihydrokaempferol or aromadendrin, MW = 288)	Adzet *et al.*, 1988*b*
3,5,7,4′,5′-pentahydroxyflavanone (dihydroquercetin or taxifolin, MW = 304)	Adzet *et al.*, 1988*b*
5,4′-dihidroxy-6,7,8-trimethoxyflavanone (dihydroxanthomicrol, MW = 346)	Adzet *et al.*, 1988*b*
5-Hydroxy-7,4′-dimethoxyflavone (7,4-dimethylapigenin, MW = 298) (298, 269, 255, 166, 138, 135, 132)[c]	Miura and Nakatani, 1989
3,5,7,3′,4′-Pentahydroxyflavone (quercetin, MW = 302)	Morimitsu *et al.*, 1995 Picuric-Jovanovic *et al.*, 1995

Ion molecular and main fragments (m/z (%)) of the mass spectra taken from [a]Wiley library (Wiley, 1990), [b]Van den Broucke *et al.* (1982) and [c]Miura and Nakatani (1989).

the food industry but also of compounds with antioxidant activity. Among these can be cited, in addition to compounds with phenolic groups such as flavonoids, vitamin E, vanillin, eugenol, thymol, carvacrol or loliolide, compounds with an ethylidene side chain able to form stable allylic tertiary free radicals (Yan and White, 1990; Tsimidou and Boskou, 1994) such as linalyl, neryl and geranyl esters.

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