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Analysis of volatile components of *Lavandula luisieri* L. by direct thermal desorption–gas chromatography–mass spectrometry

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Abstract

Volatile components of wild samples of *Lavandula luisieri* collected in Central and Southern Spain have been analyzed by direct thermal desorption coupled to gas chromatography–mass spectrometry (DTD–GC–MS). This method requires only 10–20 mg of dry sample, allowing to obtain qualitative and quantitative results from different plant parts such as flowers and leaves. Average volatile yield calculated from 51 individual plants was higher for leaves (9.7 mg g^{-1}) than for flowers (2.9 mg g^{-1}). Samples presented a high variation in their yield and composition. Major components were camphor and 1,8-cineole (up to 80.9 and 76.7% in leaves; 87.8 and 85.2% in flowers, respectively); however, these compounds were not detected in several samples. Other major component (up to 60% in flowers and leaves) was 2,3,5,5-tetramethyl-4-methylene-2-cyclopenten-1-one. Multivariate analysis was applied to quantitative data from nine selected compounds in order to show the presence of several patterns in plant composition which were only partially related to the site of collection.

Keywords: *Lavandula luisieri*; Thermal desorption; Volatile organic compounds; Necrodanes

1. Introduction

Lavandula luisieri (Rozeira) Riv.-Mart. is an aromatic *Labiatae* endemic to the Iberian Peninsula, common in the South of Portugal and in the Southwest of Spain. Although the essential oils of other *Lavandula* species present importance in the fragrance industry, volatile components of *L. luisieri* have received little attention. Previous studies have shown in *L. luisieri* the presence of several compounds also present in other *Lavandula* species such as 1,8-cineole, lavandulol, linalool and their acetates, in addition to a series of compounds with a 1,2,2,3,4-pentamethylcyclopentane (necrodane) structure [1,2]. These necrodane derivatives had only been previously found in the defensive secretion of a beetle (*Necrodes surinamensis*) [3].

As a previous step in the evaluation of the bioactive potential of isolated components from *L. luisieri*, a study on the distribution of the volatile components in *L. luisieri* wild plants has been carried out. Gas chromatography coupled to mass spectrometry (GC–MS) is the most common technique used for the analysis of volatile components, since it provides qualitative and quantitative data for complex mixtures such as those usually present in natural products. A separation step is, however, required in plant analysis, in order to obtain a volatile fraction suitable to be injected.

Methods based on distillation and extraction have been used for this purpose, but direct thermal desorption (DTD) presents important advantages: it can be directly coupled to gas chromatography and mass spectrometry [4,5]; it requires a small sample amount, allowing to analyze different parts (leaves and flowers) of an individual plant and it is a rapid method, which can afford data from a number of samples high enough to draw statistical conclusions. Chromatographic profiles of plant volatile fractions obtained by steam distillation and direct thermal desorption are similar; recovery of both low volatility and thermally labile compounds have been found to be better in thermal desorption [4].

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2. Experimental

2.1. Plant material

Lavandula luisieri (Rozeira) Riv.-Mart. samples were collected in Spain in June 2000 at the late flowering stage, in two sampling areas (T, South of Toledo province and S, North of Seville province), and under botanical surveillance. Samples (flowers and leaves) were taken from 51 individual plants and left to dry at room temperature.

A sample prepared from leaves of a single plant, ground and homogenized in a mechanical blender, was used for the evaluation of method dispersion.

2.2. Direct thermal desorption fractionation of volatile compounds

Volatile fractionation was carried out by using an ATD 400 thermal desorber (Perkin-Elmer, Norwalk, CT, USA). Cuttings of dry plant samples (10–20 mg) were introduced into a PTFE tube (52 mm × 4 mm i.d.) which was then placed into a stainless steel desorption cartridge (89 mm × 4.5 mm i.d. × 6.5 mm o.d.). Volatile compounds were desorbed under a 20 ml min^{-1} helium flow at 180 °C for 15 min and then cryofocused on a Tenax TA trap at −30 °C. This trap was heated to 320 °C at ∼40 °C s^{-1}, remaining at the maximum temperature for 4 min. The desorbed volatiles were transferred to the GC column through a fused-silica line heated at 225 °C. Inlet and outlet split flows were 50 ml min^{-1}.

2.3. Gas chromatography–mass spectrometry

The ATD 400 was connected to a GC 8000 gas chromatograph (Fisons, Milan, Italy) coupled to an MD 800 mass detector (Fisons, Manchester, UK). A methyl silicone SPB-1 column (27 m × 0.25 mm i.d., 0.25 μm film thickness) (Supelco Inc., Bellefonte, PA, USA) was temperature programmed from 60 to 180 °C (at 3 °C min^{-1}) and then to 250 °C (at 8 °C min^{-1}) for 5 min. Helium at ∼1 ml min^{-1} was used as carrier gas.

Mass spectra were recorded in the electron impact (EI) mode at 70 eV, scanning the *m*/*z* 38–350 range. Interface and source temperature were 250 and 200 °C, respectively. Data acquisition and data processing were carried out using the MassLab software, version 1.4 (Finnigan, Manchester, UK).

2.4. Qualitative and quantitative analysis

Peaks in the TIC (total ion current) profiles for both flower and leaf samples were characterized or tentatively identified from their mass spectral data by using US National Institute of Standards and Technology (NIST) and Wiley mass spectrometry libraries [6,7]. Identifications were confirmed from their chromatographic retention by using linear Kovats retention indices or standard compounds when available.

Percent concentration values were directly calculated from TIC peak areas. Semiquantitative values were obtained by using 2-dodecanone (Fluka, Buchs, Switzerland) as internal standard (10 μl of a 0.1076 mg ml^{-1} pentane solution was added to the dry samples).

2.5. Data processing

Quantitative results were processed by using the 7M (stepwise discriminant analysis (SDA)) program in the BMDP software for personal computers [8].

3. Results

3.1. Qualitative results

Flowers and leaves from the 51 collected individual plants of *L. luisieri* were submitted to DTD–GC–MS analysis. Volatile compounds characterized in both flowers and leaves are listed in Table 1. Compounds marked "*" in the second column of this table have been previously reported in other studies on *L. luisieri* essential oils [1,2]. In some cases (marked "#" in the second column of Table 1), the compound listed in Table 1 could not be unequivocally identified, and mass spectral data were used to propose a general structure or to assign an elemental composition.

Most of compounds determined by DTD–GC–MS in *L. luisieri* were monoterpenes. 1,8-Cineole, fenchone and camphor (marked respectively as A, B and C in Table 1), which appeared among the major compounds in many samples, had been previously found in essential oils of *L. luisieri* at a low concentration and in other *Lavandula* species as major components [1,9–11].

Although necrodols and their acetates had been reported as important components in *L. luisieri* [1,2], *trans*-α-necrodyl acetate (E) and *cis*-α-necrodyl acetate (F) appeared in the studied samples as minor compounds. *trans*-α-necrodol was only present in a single plant, and in a very small amount. Steam distillation, carried out using pooled plant samples, also produced a fraction with a low presence of necrodol acetates and alcohols.

Mass spectrum of compound D (see Table 1) showed ions at *m*/*z* 150 (molecular ion, 50% relative intensity), 107 (base peak, 100% relative intensity) and 135 (65% relative intensity). Molecular weight and fragmentation were compatible with a $C_{10}H_{14}O$ cyclic ketone structure. Isolation of this compound from an essential oil obtained by steam distillation was carried out by Lavoine-Hanneguelle and Casabianca [12], who used RMN data for its structural determination as 2,3,5,5-tetramethyl-4-methylene-2-cyclopenten-1-one.

Other compounds presented mass spectral features which suggested a cyclic terpene ketone structure. Most of them were minor components, and it was very difficult to obtain structural information from their mass spectra, other

Table 1
Compounds tentatively identified on the basis of their mass spectrum and Kovats retention indices in the *L. luisieri* volatile fraction (flowers and leaves) obtained by DTD

Kovats retention indices	Compound
946	α-Pinene*
946	$C_{10}H_{14}$#
954	Camphene*
956	$C_8H_{14}O$#
999	$C_8H_{10}O_2$#
1005	*p*-Cymene*
1007	β-Phellandrene
1018	1,8-Cineole* (A)
1049	$C_9H_{14}O$#
1057	*cis*-Linalool oxide (furanyl ring)
1061	$C_9H_{14}O$#
1065	Fenchone* (B)
1071	*trans*-Linalool oxide (furanyl ring)
1072	*p*-Cymenene
1087	$C_9H_{14}O$#
1092	$C_9H_{12}O$#
1124	Camphor* (C)
1135	*trans*-α-Necrodol*
1160	*cis*-Linalool oxide (pyranyl ring)
1168	*trans*-Linalool oxide (pyranyl ring)
1171	2,3,5,5-Tetramethyl-4-methylene-2-cyclopenten-1-one (D)
1174	Hotrienol
1206	$C_{10}H_{14}O$#
1206	$C_{11}H_{16}O$#
1267	Bornyl acetate
1270	*trans*-α-Necrodyl acetate* (E)
1275	Lavandulyl acetate*
1277	Lyratyl acetate
1279	$C_{10}H_{14}O_2$#
1287	*cis*-α-Necrodyl acetate* (F)
1340	$C_{10}H_{16}O_2$#
1348	$C_{10}H_{16}O_2$#: see text (G)
1358	$C_{10}H_{14}O_2$#
1517	$C_{12}H_{18}O_3$#: see text (H)
1580	Viridiflorol*
1625	Muurolol
1651	Guaiazulene
1651	Azulol
1653	Norcadinenone#
1848	Hydroxycadinenone# ($C_{15}H_{24}O_2$: see text) (I)
1856	$C_{15}H_{26}O_2$#

Structural information or elemental composition for compounds marked (#) was obtained from mass spectral data.

than their molecular weight and their elemental composition; some of them are listed in Table 1.

Compounds G and H in Table 1 appeared as major components in several samples which usually also had high amounts of D. G mass spectrum showed a molecular ion at *m/z* 168 (25%), and fragments at *m/z* 107 (100%), *m/z* 123 (85%), *m/z* 135 (70%), *m/z* 138 (65%) and *m/z* 150 (40%). Retention and mass spectral data were compatible with a $C_{10}H_{16}O_2$ structure such as that resulting of including a hydroxyl group in D. Molecular ion of H appeared at *m/z* 210 (10%) and the most important fragments were *m/z* 135 (100%), *m/z* 107 (75%), *m/z* 43 (40%) and *m/z* 150 (40%). The structure of H could correspond to the $C_{12}H_{18}O_3$ acetate of an hydroxylated ketone such as G.

A high number of minor compounds in the 1200–1600 retention index range appeared to be monoterpene alcohols, ketoalcohols or their acetates. Several compounds with a sesquiterpene structure appeared in small amounts at retention indices higher than 1600. Although in some cases their mass spectra closely match those appearing in mass spectral libraries [6,7], their identifications in Table 1 should always be considered as tentative. Among these compounds, the highest amount corresponded to I ($C_{15}H_{24}O_2$), with *m/z* 175 (100%) and *m/z* 193 (60%) as main fragments and a molecular ion at *m/z* 236, which appears listed in Table 1 as hydroxycadinenone.

3.2. Quantitative results

From the compounds listed in Table 1, nine *L. luisieri* volatile components were selected for studying the distribution of their quantitative values. 1,8-Cineole (A), fenchone (B) and camphor (C) were chosen since they were present as major components in some of the collected samples. These compounds are also present in high concentrations in other *Lavandula* species, especially in *L. stoechas*, *L. pedunculata* and *L. sampaioana*, included with *L. luisieri* in Sect. Stoechas of genus *Lavandula* [1,9–11]. D, G and H, present in relatively high amounts in the studied *L. luisieri* samples, have not been found in other *Lavandula* species.

Although lower concentrations were found for *trans*-α-necrodyl acetate (E) and *cis*-α-necrodyl acetate (F), they were also selected for quantitative analysis, as one of the objectives of this work was to study their distribution in *L. luisieri* plants. Compound I, tentatively identified as a hydroxycadinenone, was also selected as the sesquiterpene which appeared in the highest amount.

Tables 2 and 3 summarize the quantitative data for the volatile composition from the 51 *L. luisieri* leaf and flower samples, respectively. Results (minimum, maximum and

Table 2
Quantitative results for major volatile components of *L. luisieri* leaves obtained by DTD–GC–MS

Compound	Leaves					
	Absolute concentration ($mg\,g^{-1}$)			Relative concentration (%)		
	Min	Max	Mean	Min	Max	Mean
A	0.00	12.32	2.36	0.00	76.68	23.60
B	0.00	1.16	0.14	0.00	18.21	2.40
C	0.00	14.23	1.94	0.00	80.91	16.40
D	0.39	11.43	2.27	9.89	60.93	24.50
E	0.00	0.18	0.03	0.00	2.87	0.38
F	0.00	0.17	0.05	0.02	1.99	0.61
G	0.11	2.58	0.76	1.64	16.78	8.40
H	0.03	5.24	1.99	0.33	52.61	22.38
I	0.00	0.66	0.11	0.00	6.17	1.34

See Table 1 for identification of compounds.

Table 3
Quantitative results for major volatile components of *L. luisieri* flowers obtained by DTD–GC–MS

Compound	Flowers					
	Absolute concentration ($mg\,g^{-1}$)			Relative concentration (%)		
	Min	Max	Mean	Min	Max	Mean
A	0.00	5.96	0.69	0.00	85.18	19.92
B	0.00	2.45	0.14	0.00	64.22	6.65
C	0.00	7.22	0.91	0.00	87.83	28.49
D	0.02	2.52	0.36	0.37	59.97	16.47
E	0.00	0.44	0.02	0.00	4.18	0.23
F	0.00	0.15	0.01	0.00	1.21	0.34
G	0.00	1.59	0.15	0.54	13.38	4.02
H	0.01	4.64	0.53	0.96	52.33	17.16
I	0.00	0.58	0.13	0.00	38.10	6.71

See Table 1 for identification of compounds.

mean values) are listed as absolute ($mg\,g^{-1}$) and relative (percent of quantitatively determined compounds) concentrations. Total volatile amount was higher in leaf samples, as shown by the mean absolute concentrations listed in Tables 2 and 3.

A high variation in the concentration of the volatiles studied for both flower and leaf samples was also observed. Camphor and 1,8-cineole, which were main volatile components in many leaf samples, were not detected in others. A similar behaviour was observed in flower volatiles for camphor, 1,8-cineole and fenchone.

DTD–GC–MS precision in the analysis of *L. luisieri* volatiles was estimated from the compounds previously selected for quantitation purposes, using an homogenized leaf sample. Mean (percent composition, %) and relative standard deviation (R.S.D., %) values for five replicates appear listed in columns 2 and 3 of Table 4.

Dispersion in the volatile composition of flowers and leaves from a single plant was also evaluated. Table 4 lists the percent values (mean and relative standard deviation) obtained for the nine selected compounds in the analysis ($n = 5$) of different leaves (columns 4 and 5) and flowers (columns 6 and 7) collected from a single plant.

Table 4
Percent concentration values (mean and R.S.D.) for *L. luisieri* volatile components fractionated by DTD from: a leaf sample after homogenization (columns 2 and 3), cuttings from different leaves of a single plant (columns 4 and 5) and cuttings from different flowers of a single plant (columns 6 and 7)

Compound	Homogenized leaves		Leaves		Flowers	
	Mean (%)	R.S.D. (%)	Mean (%)	R.S.D. (%)	Mean (%)	R.S.D. (%)
A	0.7	26.2	0.7	63.8	0.0	23.6
B	9.4	13.0	11.2	8.5	16.8	15.0
C	52.3	8.2	52.9	8.9	70.4	2.8
D	14.2	12.7	17.6	12.5	4.1	6.9
E	0.1	41.7	0.1	33.3	0.0	36.2
F	0.3	42.3	0.1	43.9	0.1	51.8
G	3.3	31.8	2.3	20.6	0.8	24.1
H	18.8	14.9	14.4	17.6	5.7	25.2
I	0.9	49.3	0.8	58.6	2.1	46.6

A comparison of the R.S.D. values in columns 3, 5 and 7 of Table 4 showed that dispersion of a compound was highly influenced by its relative proportion. Average R.S.D. values were higher than those found in other plant analysis by DTD–GC–MS [4]. Dispersion was roughly similar in the three columns compared, indicating that volatile components appear to be homogeneously distributed in the different plant parts.

The broad concentration range listed in Tables 2 and 3 is graphically shown in Figs. 1 and 2, where chromatographic profiles are shown for a Seville sample (leaves, Fig. 2a; flowers, Fig. 2b), rich in D, G and H, and for a Toledo sample (leaves, Fig. 1a; flowers, Fig. 1b), where the major component was camphor.

However, TIC profiles shown in Figs. 1 and 2 were not representative of the volatile composition of the samples collected in these locations. In >50% of Seville leaf and flower samples, the main component was 1,8-cineole, although D, G and H were always present. In about 50% of Toledo leaf samples, camphor was the major component as in Fig. 1, but in most of the other cases studied, its concentration decreased below 5%. 1,8-Cineole content was also highly variable for all Toledo samples. Fenchone was not detected in any of Seville leaf and flower samples and in many Toledo samples, but it was present at levels higher than 20% in some Toledo flowers.

3.3. Statistical analysis

Statistical analysis was applied to *L. luisieri* quantitative data (relative concentration for volatile components in flower and leaf samples), in order to show the presence of trends or patterns related to plant parts (flowers and leaves), collection places (Seville and Toledo), or to the existence of sample groups having a different volatile composition.

Camphor and 1,8-cineole presented the highest average concentrations (Tables 2 and 3). Fig. 3 plots the relative concentrations of these compounds in the studied leaf samples. The lower right of the plot includes a group of Toledo samples with a low presence (<15%) of 1,8-cineole and high relative amounts (between 30 and 80%) of camphor. The group at the left side of the plot, which is characterized by low concentrations (less than 10%) of camphor, includes both Toledo and Seville samples: 1,8-cineole presents a continuously variable concentration which ranges from 77% for samples at the top left of the plot, to 0% for the bottom left samples, rich in ketones D, G and H.

Fig. 4 shows for flower samples a distribution of camphor and 1,8-cineole similar to that of Fig. 3. A group of Toledo samples with high camphor contents (40–96%) appears at the bottom right of the plot. Several Toledo samples, at the centre of the plot, are characterized by intermediate (in the 20–30% range) concentration values of fenchone, camphor

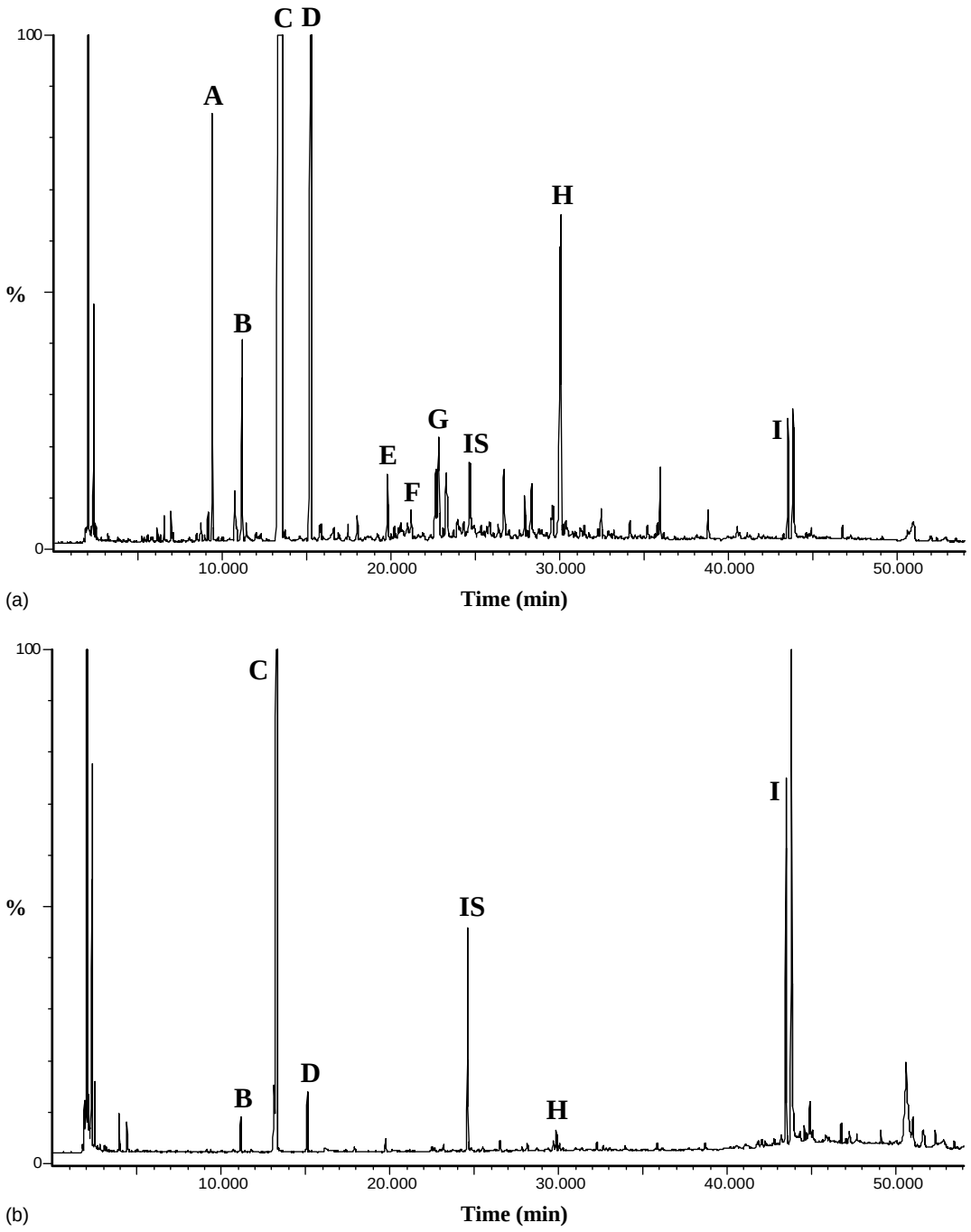

Fig. 1. TIC profiles for a *L. luisieri* single plant collected in Toledo: (a) leaves and (b) flowers. For peak identification, see Table 1.

and 1,8-cineole. Sevilla flower samples, grouped at the left of the plot, present camphor contents below 20% and variable amounts of 1,8-cineole, but some Toledo samples also showed a similar composition. Samples with high relative amounts of ketones D, G and H are, as in Fig. 3, plotted at the bottom left corner.

3.3.1. Correlation coefficients

Correlation coefficients between the concentrations of a given compound in flowers and in leaves for each individual plant were calculated in order to find possible relationships. The highest values were found for camphor ($r = 0.85$) and 1,8-cineole ($r = 0.75$).

When considering flower and leaf samples as separate groups, most correlation coefficients among the concentrations of the nine selected compounds were found to be negative or of low absolute value. The only significative *r* values (0.5–0.75) were found in both leaves and flowers for pairs of compounds belonging to the group formed by ketones D, G and H and necrodyl acetates.

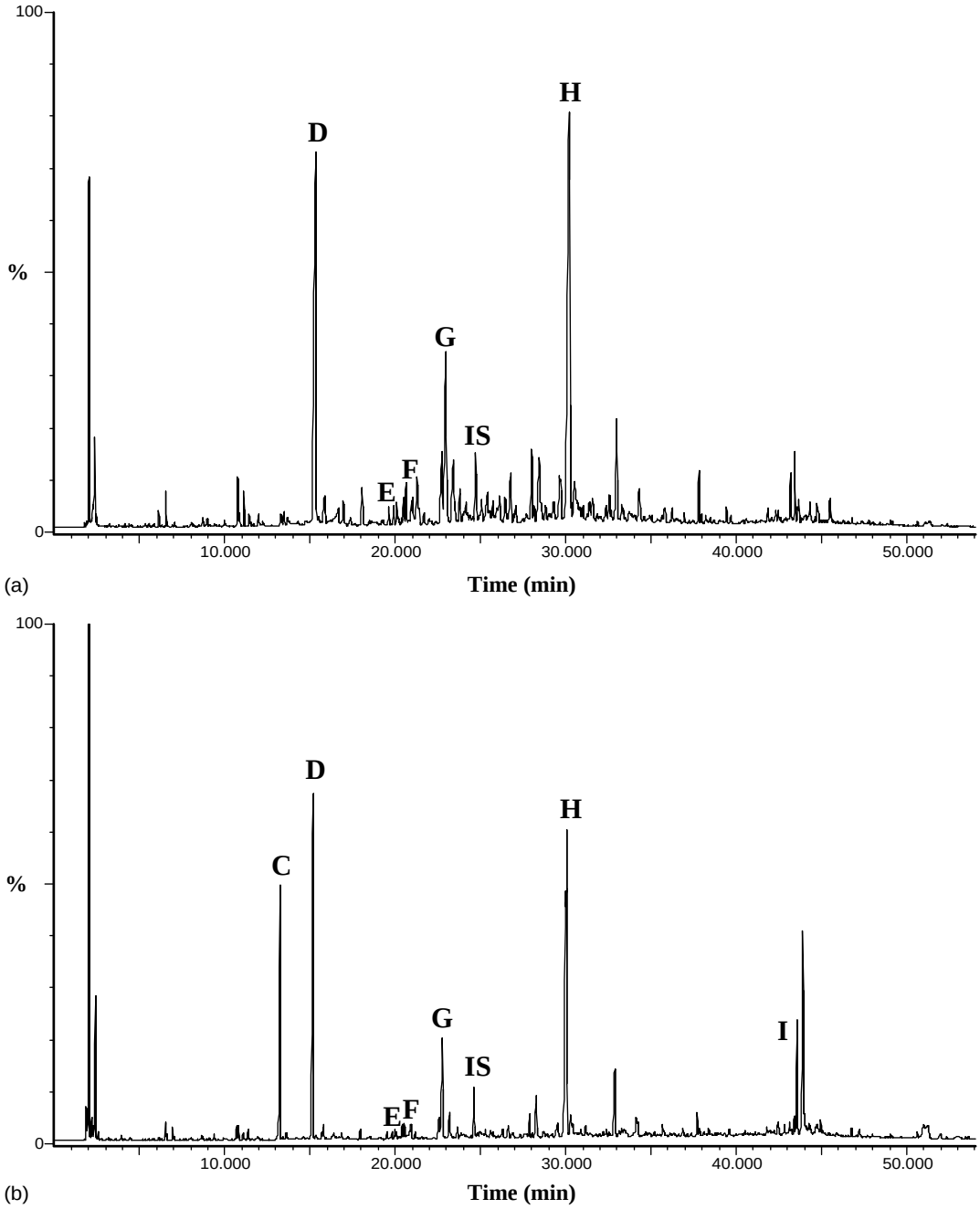

Fig. 2. TIC profiles for a *L. luisieri* single plant collected in Seville: (a) leaves and (b) flowers. For peak identification, see Table 1.

3.3.2. Discriminant analysis

Stepwise discriminant analysis was used not as a classifying tool, but in order to find if some compounds presented significant differences between their concentrations in flowers and leaves, or among Toledo and Seville samples. *F*-values for these compounds were calculated using discriminant analysis (DA) in the step 0, and are listed in Table 5.

Ketone G, which was present in higher concentration in leaves, and compound I, more abundant in flowers, appeared to be the compounds with the highest separation power; however, flowers and leaves could not be clearly distinguished from their volatile composition even when using additional compounds.

Discriminant analysis was also applied separately to the volatile concentrations of leaves and flowers, looking in each group for differences between Seville and Toledo samples. The results for leaf volatiles showed fenchone, camphor and I as the compounds with the highest discriminant power. Seville samples formed a close group characterized by very low concentrations of these compounds, but which included some similar Toledo samples.

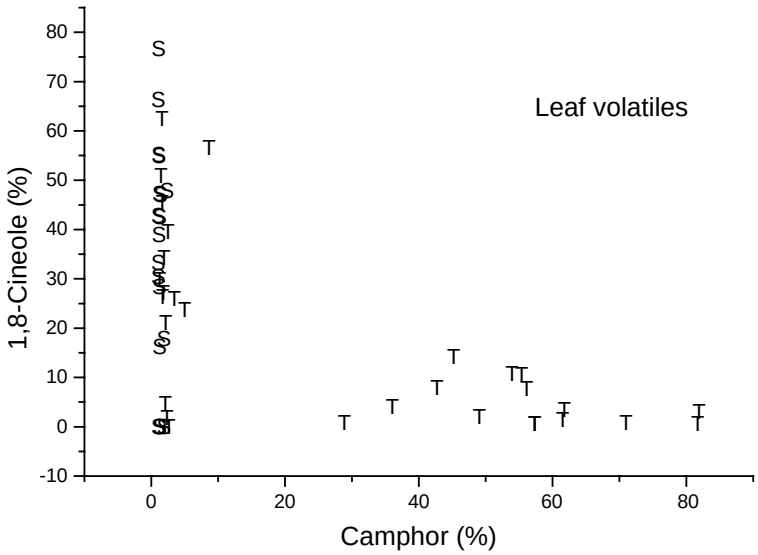

Fig. 3. Camphor vs. 1,8-cineole percent concentration plot for *L. luisieri* leaf samples. S: Seville samples; T: Toledo samples.

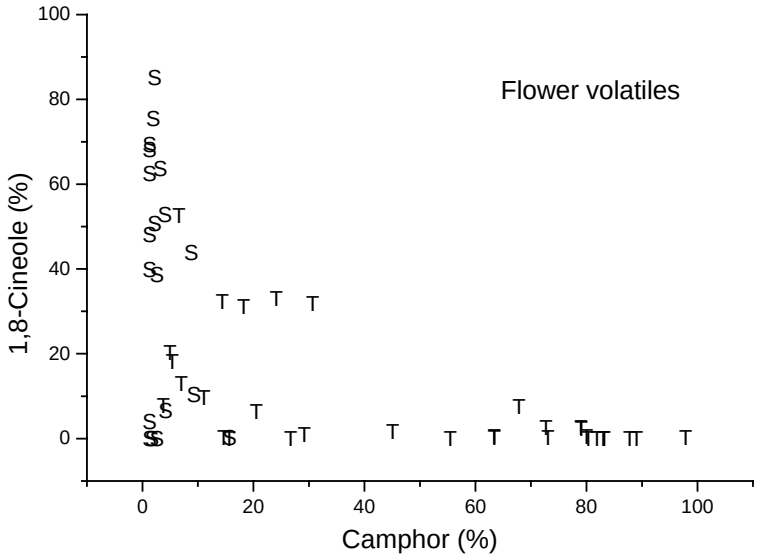

Fig. 4. Camphor vs. 1,8-cineole percent concentration plot for *L. luisieri* flower samples. S: Seville samples; T: Toledo samples.

Table 5
F-values calculated in discriminant analysis of *L. luisieri* volatile quantitative composition

Compound	*F*-values for separation between		
	Leaves–flowers	Toledo–Seville (leaves)	Toledo–Seville (flowers)
A	0.89	7.29	23.10
B	3.29	13.00	9.78
C	4.49	14.40	32.34
D	8.71	4.69	15.04
E	1.33	0.84	0.21
F	10.43	3.20	0.78
G	30.83	1.79	6.45
H	4.48	6.54	26.70
I	23.52	21.09	11.36

See text for identification of compounds A–I.

For flower samples, fenchone, camphor and compound I presented higher concentrations in Toledo samples, while the amounts of 1,8-cineole and ketones D and H were higher in those of Seville. A clear separation between Toledo and Sevilla flower samples could not, however, be achieved.

4. Discussion

Although our survey of *L. luisieri* flower and leaf samples only covered two separate zones, the range of variation found for their composition (see minimum and maximum values in Tables 2 and 3) was very broad. This variation was usually continuous, although intermediate values (between 10 and 30%) were missing for the concentration of camphor

in leaf samples (see Fig. 3). Covariation of the most important *L. luisieri* volatiles was also complex. Values of fenchone concentration seemed to be independent from those of other compounds. The major volatile compounds in most of samples were camphor or 1,8-cineole, which were present in some cases in concentrations higher than 50% and showed, for this reason, negative correlations with the rest of compounds. The range of concentration of the ketones D, G and H was smaller: these compounds, and the necrodyl acetates, presented among them positive correlation coefficients.

Camphor versus 1,8-cineole plots shown in Figs. 3 and 4 indicated that *L. luisieri* samples formed clear patterns, clustered along a broad range of concentration values instead of around an average composition. A separation between Seville and Toledo samples for both flowers and leaves was impossible since some Toledo samples presented a composition similar to those of Seville.

The volatile composition of *L. luisieri* samples seems to be the result of several methabolic pathways which act in each sample at a different extent for individual components (fenchone, camphor or 1,8-cineole) or for groups of compounds of related structure (necrodyl compounds, 2,3,5,5-tetramethyl-4-methylene-2-cyclopenten-1-one related compounds). A continuation of the present study is necessary in order to assess if the peculiar *L. luisieri* composition distribution described is the habitual one for the collection areas, or if an annual component can affect in an important extent the concentration, or even the presence, of some of *L. luisieri* volatile compounds.

Results also indicated that a chemical survey of individual plants is necessary as a previous step to the collection of *L. luisieri* samples, when the preparative isolation of some of its volatile components is pretended. Separate determination of flower and leaf volatiles seems less important, as their composition differences appear to be only quantitative, but could be of interest in other studies on the distribution of plant volatiles. Direct thermal desorption, when coupled to gas chromatography–mass spectrometry, presents as main advantages for this task the simple and fast sample preparation, the short analysis time and the small sample amount required.

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