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Volatile composition of *Catharanthus roseus* (L.) G. Don using solid-phase microextraction and gas chromatography/mass spectrometry

P. Guedes De Pinho[a,*], Rui F. Gonçalves[a], Patrícia Valentão[a], David M. Pereira[a], Rosa M. Seabra[a], Paula B. Andrade[a,*], Mariana Sottomayor[b]

[a] *REQUIMTE/Serviço de Farmacognosia, Faculdade de Farmácia da Universidade do Porto, R. Aníbal Cunha 164, 4050-047 Porto, Portugal*
[b] *IBMC-Instituto de Biologia Molecular e Celular, Universidade do Porto and Departamento de Botânica, Faculdade de Ciências, Universidade do Porto, R. Campo Alegre 823, 4150-180 Porto, Portugal*

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ABSTRACT

A total of 88 volatile and semi-volatile components were formally or tentatively identified in flowers, leaves and stems of *Catharanthus roseus* (L.) G. Don (cv. Little Bright Eye), by headspace solid-phase microextraction (HS-SPME) and by dichloromethane extraction, combined with gas chromatography–mass spectrometry (GC–MS). These include some diterpenic compounds (manool and manoyl oxides), a sesquiterpen (α-bisabolol), and some pyridine, pyrazine, indol and carotenoid derivatives. Applying multivariate analysis (principal component analysis and agglomerative hierarchic cluster analysis) to the HS-SPME–GC–MS data, it was possible to characterize each part of the vegetal material using a relative small number of compounds. Hence, flowers were richer in terpenic molecules (including limonene), α-bisabolol, methyljasmonate, *cis*-jasmone, 2-phenylethanol, phenylacetaldehyde, *trans*-2-octenal, benzylic alcohol and 2-isobutyl-3-methoxypyrazine. Leaves can be characterized by the methyl and propyl esters of fatty acids, mono- and disaturated, *trans*-phytol, carotenoid derivative compounds, hydrofarnesylacetone, methylanthranilate, manool and epi-manool oxide, while stems have high levels of volatile aldehydes, such as hexanal, octanal, *cis*-2-nonenal, *cis*-2-decenal, *cis*, *trans*-2,6-nonadienal, *trans*, *trans*-2,4-decadienal and *cis*, *trans*-2,4-decadienal. Dichloromethane extraction allowed also the identification of some alkaloid-like compounds that were not detected by HS-SPME.

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1. Introduction

Catharanthus roseus (L.) G. Don, formerly *Vinca rosea* L. (Apocynaceae), is commonly known as the Madagascar periwinkle. More than 100 different terpenoid indole alkaloids (TIAs) [1], with important pharmacological activity, like anticancer (vinblastine and vincristine), antihypertensive (ajmalicine) and sedative (serpentine) were identified [1,2]. Water extracts from *C. roseus* are used in folk medicine for preventing some diseases, such as bleeding arresting, diabetes, fever or rheumatism [3]. Furthermore, the leaves of the plants are chewed to suppress the sensations of hunger and fatigue [1].

In spite of all the attention focused on *C. roseus*, the characterization of natural products other than alkaloids in this plant remains scarce. A few recent reviews concerning the occurrence of phenolics in this species [4–6] illustrate how little has been done in the characterization of this group of compounds in *C. roseus* and, moreover, most of the work reported has been done with cell cultures, where the metabolism of natural products is quite poor compared with the differentiated plant body.

Two previous screenings using hydrodistillation and organic solvents to extract volatiles from *C. roseus* leaves and flowers have been reported, enabling the identification of several types of compounds, namely alkanes, aldehydes, ketones, fatty acids and their esters, terpenoids, phenylpropanoids and alcohols [7,8]. These two extraction methodologies have traditionally been applied for essential oil extraction from plant material [7–9], although they present some shortcomings, such as losses of volatile compounds, low extraction efficiency and long extraction time. Also, high temperatures and water can cause degradation or chemical modifications of volatile constituents [10].

In recent years, the most frequent analytical techniques applied in the extraction and concentration of volatile compounds from aromatic and medicinal plants are those based on headspace analysis (HS). Among the headspace methods, the solid-phase microextraction (SPME) constitutes a reliable tool for the analysis of organic volatile and also semi-volatile compounds [11–13].

The aim of this work was to extend the knowledge of volatile compounds of *C. roseus* by using the HS-SPME technique directly

* Corresponding authors. Tel.: +351 222078922; fax: +351 222003977.
E-mail addresses: pguedes@ff.up.pt (P. Guedes De Pinho), pandrade@ff.up.pt (P.B. Andrade).

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into the headspace of fresh flowers, leaves and stems, and in the headspace of the aqueous lyophilized extract of the leaves. Less volatile compounds were also determined by the use of organic solvents. The application of these two extraction techniques constitutes an interesting and novel screening of volatile and semi-volatile compounds of *C. roseus*, additionally allowing the discrimination of the distinct plant materials.

2. Experimental

2.1. Standards and reagents

Reference compounds were purchased from various suppliers: 4-decanol (used as internal standard) was purchased from Acrōs Organics (Geel, Belgium), caproic acid ethyl ester, eugenol, geranylacetone, citronellol, 2,6,6-trimethyl-1-cyclohexene-1-acetaldehyde, 80% containing β-cyclocitral, 2-isobutyl-3-methoxy-pyrazine, 2-isopropyl-3-methoxypyrazine, octanal, hexanal, *trans*-2-octenal, *trans*-2-nonenal, *cis*-3-hexen-1-ol, *trans*-hexen-1-ol, *trans*-2-nonen-1-ol, *a*-bisabolol, 6-methyl-5-hepten-2-ol, 2-decen-1-ol, *trans*-2,6-nonadienal, *trans*, *trans*-2,4-decadienal and 6-methyl-5-hepten-2-one were from Sigma (St. Louis, MO, USA); benzylic alcohol, benzaldehyde, phenylacetaldehyde, β-ionone, methional, 2-octen-1-ol, methyljasmonate, manool, 1-octen-3-one and γ-decalactone were obtained from SAFC (Steinheim, Germany); R-(+)-limonene, 1-hexanol, 2-phenylethanol and (−)-menthol were from Fluka (Buchs), Switzerland. Dichloromethane (≥99.8% pure) was obtained from Fluka (Buchs, Switzerland). Ethanol (pure grade ≥99.9%) was from Merck (Darmstadt, Germany). Anhydrous sodium sulphate was purchased from Panreac Química Sau (Barcelona, Spain). The water was treated in a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Samples

2.2.1. Fresh material

Flowers, leaves and stems of *C. roseus* (L.) G. Don cv. Little Bright Eye were collected after authentication by Mariana Sottomayor (IBMC/Faculty of Science of Porto University). Voucher specimen of plant material is available in Pharmacognosy Laboratory at Faculty of Pharmacy of Porto University.

2.2.2. Aqueous extract

1.5 g of dried leaves were boiled for 20 min in 300 mL of water and filtered over a Büchner funnel. The resulting extract was then lyophilized in a Labconco 4.5 Freezone apparatus (Kansas City, MO, USA). The lyophilized extract was kept in an dessicator, in the dark.

2.3. Sample preparation for GC–MS analysis

Two methodologies, SPME technique and organic solvents, were used concerning the determination of the total amount of volatile and semi-volatile and other non-volatile compounds, respectively. HS-SPME was applied to fresh plant (to identify the most volatile compounds) and to lyophilized extract (to determine the less volatile components). The dichloromethane extraction was performed to determine the non-volatile compounds.

2.4. Extraction methodologies

2.4.1. SPME technique SPME fibres

Several commercial fibres can be used to extract volatiles. According to bibliography, recommendations of supplier (Supelco, Bellefonte, PA, USA) and to our own knowledge [11] three of them are most adaptable to the intended compounds and to the matrix under study. The fibres used were coated with different stationary phases and various film thickness: Black–Carboxen TM/polydimethylsiloxane (CAR/PDMS), 75 μm; Orange–Carbowax/Divinylbenzene (CW/DVB), 65 μm; Blue–Divinylbenzene/PDMS (DVB/PDMS), 50/30 μm. They were conditioned by inserting them into the GC injector; temperature and time were used according to the procedure recommendation of Supelco: 300 °C for 1 h, 220 °C for 30 min, and 250 °C for 30 min, respectively.

2.4.2. Headspace solid-phase microextraction (HS-SPME) (fresh plant). Qualitative and semi-quantitative SPME analysis

Divinylbenzene/PDMS (DVB/PDMS), 50/30 μm fibre was used. In this case only fresh flowers, leaves and stems were studied. Samples were stirred at 600 rpm, at 50 °C for 15 min. The fibre was then exposed to the headspace for 60 min, with agitation (800 rpm). Afterwards the fibre was pulled into the needle sheath and the SPME device was removed from the vial and inserted into the injection port of the GC system for thermal desorption. After 5 min the fibre was removed and conditioned in another GC injection port for 15 min at 250 °C. The same procedure was used to test CAR/PDMS and CW/DVB fibres.

Compounds were identified by comparing the retention times of the chromatographic peaks with those of authentic compounds run under the same conditions, and by comparison of the retention indices (as Kovats indices) with the literature data. The comparison of MS fragmentation pattern with those of pure compounds and mass spectrum database search was performed using the National Institute of Standards and Technology (NIST) MS 05 spectral database. Confirmation was also conducted using the laboratory built MS spectral database, collected from chromatographic runs of pure compounds performed with the same equipment and conditions. The relative amounts (RAs) of individual components are expressed as percent peak areas relative to total peak area.

2.4.3. Headspace solid-phase microextraction (SPME) (lyophilized aqueous extract)

Approximately 0.2 g of lyophilized aqueous leaves extract was dissolved in 5 mL of a 10% ethanol solution in a 15 mL vial, and 0.5 g of anhydrous sodium sulphate was added to favour the release of analytes from the matrix. It was then sealed with a polypropylene hole cap and PTFE/silicone septa (Supelco, Bellefonte, PA, USA). The mixture was then magnetically stirred at 760 rpm, at 55 °C, for 5 min. The fibre was then exposed to the headspace for 60 min, with agitation (800 rpm). Afterwards, the fibre was pulled into the needle sheath and the SPME device was removed from the vial and inserted into the injection port of the GC system for thermal desorption. After 5 min the fibre was removed and conditioned in another GC injection port for 15 min at 250 °C. This methodology is adapted from previous work performed in wine matrix [11].

2.4.4. Dichloromethane extraction

Approximately 200 mg of each part of *C. roseus* plant were directly extracted with 15 mL of dichloromethane; 50 μL of 4-decanol (1.26 mg/L) alcoholic solution was added as an internal standard. The sample suspended in dichloromethane was magnetically stirred at 760 rpm for 4 h and then filtered through a Bückner filter under vacuum. Afterwards the extract was dehydrated over 0.5 g of anhydrous sodium sulphate and concentrated under nitrogen gas to obtain a final volume of 0.3 mL [12].

2.5. Gas chromatography–mass spectrometry analysis

Dichloromethane extracts were analyzed using a Varian CP-3800 gas chromatograph (USA) equipped with a VARIAN Sat-

Table 1
Identification or tentative identification of alcohol and ketone compounds in *C. roseus*.

Number	Retention time/RI values	Quantification ions	EIMS fragmentation (relative intensity, %)	Name	ID[a] (fit/Rfit)	RA[b] (%) leaves	RA (%) stems	RA (%) flowers
1	3.68/734	55; 71	55 (100); 70 (49); 71 (19); 57 (14); 56 (9); 69 (7); 53 (4); 51 (3); 54 (2); 50 (2)	3-Methyl-1-butanol[(a)]	S[c], RI[d], MS[e]	0.15	0.61	0.08
2	3.74/744	56; 70	56 (100); 57 (94); 55 (89); 70 (72); 71 (14); 58 (12); 69 (11); 53 (10); 85 (7); 50 (6)	2-Methyl-1-butanol[(a)]	S[c], RI, MS	0.03	0.26	0.10
3	4.23/766	55; 70	55 (100); 70 (42); 57 (26); 56 (15); 71 (10); 69 (9); 53 (6); 50 (4); 67 (4); 54 (4)	1-Pentanol[(a)]	RI, MS	1.64	0.24	0.85
4	4.28/783	57; 67	57 (100); 67 (32); 55 (28); 68 (28); 53 (13); 69 (11); 71 (9); 51 (7); 70 (7); 56 (7)	*cis*-2-Penten-1-ol[(a)]	RI, MS	0.26	0.07	0.03
5	6.21/849	55; 67; 82	67 (100); 82 (36); 55 (34); 83 (21); 69 (14); 81 (12); 53 (10); 57 (9); 68 (8); 54 (7)	*cis*-3-Hexen-1-ol[(a)]	S[c], RI, MS	4.17	0.57	1.07
6	6.30/851	57; 67	67 (100); 55 (44); 82 (30); 69 (24); 83 (17); 57 (15); 53 (11); 54 (11); 68 (9); 81 (8)	*trans*-3-Hexen-1-ol[(a)]	S[c], RI, MS	–	0.39	–
7	6.56/884	56; 69	56 (100); 55 (57); 69 (48); 57 (9); 85 (7); 83 (5); 53 (5); 54 (4); 84 (3); 70 (3)	1-Hexanol[(a)]	S[c], MS	0.62	0.48	0.27
8	11.46/1050	79; 108	79 (100); 77 (61); 108 (39); 57 (39); 107 (29); 55 (29); 82 (27); 83 (19); 51 (18); 56 (17)	Benzyl alcohol[(a)]	S[c], MS	0.34	–	4.05
9	12.26/1060	79; 107	79 (100); 107 (58); 77 (52); 78 (17); 51 (14); 50 (8); 122 (8); 80 (7); 105 (8); 108 (5)	1-Phenylethanol[(a)]	S[c], RI, MS	–	1.89	6.36
10	12.37	57; 67; 81	59 (100); 55 (70); 83 (50); 41 (49); 43 (23); 101 (19); 57 (15); 58 (12); 44 (12); 42 (8)	*trans*-2-Octen-1-ol[(a)]	S[c], MS	–	–	1.42
11	13.55	57; 81; 97	57 (100); 43 (56); 29 (46); 72 (28); 41 (27); 55 (23); 58 (16); 85 (12); 71 (9); 54 (8)	2-Nonen-1-ol[(a)]	S[c], MS	1.02	35.30	3.76
12	13.75	95	95 (100); 81 (96); 71 (85); 58 (31); 67 (25); 55 (23); 110 (21); 85 (21); 69 (17); 53 (16)	6-Methyl-2-hepten-2-ol[(a)]	S[c], MS	0.32	–	3.30
13	13.81	91	91 (100); 92 (61); 65 (20); 63 (72); 122 (71); 89 (58); 51 (52); 93 (48); 77 (4); 78 (4)	2-Phenylethanol[(a)]	S[c], MS	–	–	6.62
14	16.52	81; 95	57 (100); 67 (92); 55 (91); 81 (86); 82 (83); 83 (66); 70 (58); 68 (56); 95 (54); 56 (45)	*trans*-2-Decen-1-ol[(a)]	S[c], MS	0.71	16.20	1.65
				$\sum$ of alcohol compounds		9.25	56.01	29.56
15	9.96	55; 83	55 (100); 70 (78); 27 (53); 43 (28); 41 (19); 29 (17); 39 (13); 28 (9); 42 (9); 83 (8)	1-Octen-3-one[(a)]	S[c], MS	–	–	1.84
16	9.92/985	93; 108	43 (100); 41 (46); 69 (34); 55 (33); 108 (28); 58 (17); 111 (17); 68 (15); 39 (13); 71 (13)	6-Methyl-5-hepten-2-one	RI, MS	0.40	1.40	0.71
17	21.69/1338	79; 122; 149; 164	79 (100); 91 (63); 131 (55); 122 (54); 93 (53); 77 (48); 149 (44); 135 (40); 107 (33); 67 (31)	*cis*-Jasmone[(a)]	RI, MS	–	–	0.58
18	24.21	71; 99	71 (100); 99 (87); 55 (36); 70 (15); 69 (14); 56 (14); 68 (10); 171 (8); 96 (8); 84 (8)	γ-Decalactone[(a)]	S[c], MS	3.07	–	–
19	24.67	83; 111	83 (100); 55 (53); 111 (36); 112 (21); 82 (13); 67 (12); 81 (12); 121 (11); 99 (11); 69 (11)	3,6-Dimethyl-5-octen-2-one[(a)]	MS (64,7/70,5)	0.39	–	–
20	31.85/1848	95; 109	95 (100); 58 (76); 109 (76); 71 (63); 81 (51) 57 (47); 85 (44); 55 (39); 59 (39); 69 (32)	Hexahydrofarnesylacetone	RI, MS	4.94	1.17	0.26
				$\sum$ of ketone compounds		8.80	2.57	3.38

[a] Identification method (fit/retrofit values, %); compound name[(a)]—identified for the first time.
[b] Relative area in percentage.
[c] Identified by comparison with reference compound.
[d] Tentatively identified by retention indices on HP-5 capillary column.
[e] Tentatively identified by NIST05.

urn 4000 mass selective detector (USA) and a Saturn GC/MS workstation software version 6.8. The column used for quantification analysis was VF-5ms 30 m × 0.25 mm × 0.25 μm (FactorFour) from VARIAN. Another column, STABILWAX-DA (60 m × 0.25 mm, 0.25 μm) fused silica (Restek, USA), was employed in order to certify the identity of some compounds. The injector port was heated to 220 °C. The injection was done in split mode, with split ratio of 1/40. The carrier gas was Helium C-60 (Gasin, Portugal), at 1 mL/min,

Table 2
Identification or tentative identification of aldehyde and ester compounds in *C. roseus*.

Number	Retention time/RI values	Quantification ions	EIMS fragmentation (relative intensity, %)	Name	ID[a] (fit/Rfit)	RA[b] (%) leaves	RA (%) stems	RA (%) flowers
21	2.65/643	58; 71	58 (100); 71 (38); 57 (33); 69 (28); 85 (14) 53 (8); 87 (8); 55 (6); 50 (6); 67 (6)	3-Methylbutanal[(a)]	RI[c], MS[d]	–	–	0.70
22	2.73	58	58 (100); 57 (90); 55 (9); 53 (6); 50 (6); 56 (6); 71 (5); 51 (3); 69 (3); 59 (3)	Pentanal[(a)]	S[e], MS	–	–	0.18
23	3.10	58	58 (100); 57 (63); 81 (18); 55 (14); 69 (11); 53 (11); 87 (9); 67 (8); 50 (7); 96 (6)	2-Methylbutanal[(a)]	MS (73,4/79,0)	–	–	0.18
24	4.92/803	56; 83	56 (100); 57 (75); 67 (37); 72 (32); 55 (31) 82 (28); 58 (16); 71 (16); 83 (11); 53 (9)	n-Hexanal[(a)]	S[e], RI, MS	0.05	1.20	0.55
25	7.67	76; 104	48 (100); 104 (51); 47 (43); 76 (33); 45 (28); 61 (28); 27 (28); 29 (17); 56 (13); 28 (12)	Methional[(a)]	S, MS	–	–	0.44
26	9.31	77; 105	105 (100); 77 (49); 106 (29); 51 (18); 107 (15); 78 (14); 50 (9); 74 (5); 52 (5); 76 (3)	Benzaldehyde	S, MS	18.64	2.57	2.42
27	10.48/979	67; 81; 95	67 (100); 56 (93); 57 (81); 69 (74); 55 (78); 82 (63); 81 (55); 68 (51); 84 (36); 95 (24)	Octanal	S[e], RI, MS	0.16	4.06	0.51
28	11.77/1056	91	91 (100); 92 (42); 65 (26); 63 (8); 89 (6); 51 (5); 50 (5); 93 (4); 57 (4); 62 (4)	Phenylacetaldehyde[(a)]	S[e], RI, MS	0.78	4.23	11.73
29	12.15/1076	70; 93	55 (100); 70 (76); 83 (71); 93 (61); 67 (51); 57 (48); 69 (48); 91 (33); 82 (32); 79 (23)	*trans*-2-Octenal	S[e], RI, MS	0.14	0.85	0.94
30	15.18	55; 70; 93	55 (100); 70 (82); 83 (77); 69 (62); 81 (50) 57 (45); 67 (42); 56 (36); 93 (35); 84 (23)	*cis*-2-Nonenal	MS (81,8/86,7)	–	2.30	0.75
31	18.13/1251	83; 70	55 (100); 70 (93); 83 (81); 81 (77); 57 (50) 69 (49); 67 (48); 95 (43); 68 (37); 79 (34)	*cis*-2-Decenal	RI, MS	0.26	1.2	0.31
32	20.82	121; 93	121 (100); 122 (61); 65 (49); 93 (34); 60 (14) 63 (13); 73 (13); 55 (13); 85 (11); 66 (10)	*p*-Hydroxy-benzaldehyde[(a)]	MS (71,0/83,2)	1.32	–	0.13
				$\sum$ of aldehyde Compounds		21.61	16.41	19.08
33	16.87	88; 99	88 (100); 99 (39); 71 (26); 87 (23); 59 (17); 144 (17); 55 (12); 89 (6); 111 (4); 72 (4)	Ethyl hexanoate[(a)]	S[e], MS	5.46	–	2.24
34	27.20	129; 200	61 (100); 60 (84); 200 (50); 57 (50); 55 (47); 201 (40); 73 (38); 129 (30); 69 (30); 87 (29)	Isopropyllaurate[(a)]	MS (75,9/78,9)	0.13	0.75	0.11
35	27.37	83; 93; 151	83 (100); 93 (63); 79 (56); 95 (55); 91 (54); 67 (53); 133 (49); 151 (47); 81 (44); 55 (41)	Methyljasmonate[(a)]	S[e], MS	–	0.37	0.63
36	31.50/1827	129; 228	61 (100); 228 (58); 60 (58); 55 (55); 57 (45); 229 (42); 129 (31); 71 (28); 69 (27); 73 (26)	Isopropyl myristate[(a)]	RI, MS	0.40	0.43	0.06
37	33.56/1918	87; 227; 270	74 (100); 87 (83); 55 (38); 143 (35); 75 (28); 270 (24); 69 (20); 227 (19); 57 (17); 171 (16)	Palmitate acid methyl ester	RI, MS	1.42	–	0.14
38	34.86/1975	88; 157, 284	88 (100); 101 (56); 43 (36); 41 (27); 55 (23); 29 (23); 57 (22); 89 (14); 69 (13)	Palmitic acid ethyl ester[(a)]	RI, MS	5.09	0.74	0.26
39	35.40	61; 257; 256	61 (100); 256 (77); 57 (64); 60 (63); 55 (56); 73 (45); 83 (42); 257 (36); 71 (33); 69 (32)	Isopropyl palmitate[(a)]	MS (71,6/75,0)	–	–	0.20
40	36.84	79; 95; 107; 92	79 (100); 67 (62); 95 (58); 93 (55); 81 (54); 80 (35); 91 (34); 55 (30); 94 (27); 107 (24)	Methyl linolenate[(a)]	S[e], MS	0.16	–	–
41	36.84/2101	67; 81; 95	67 (100); 81 (75); 55 (59); 95 (51); 54 (42); 68 (37); 82 (36); 69 (35)	Linolenic ethyl ester[(a)]	S[e], RI, MS	0.16	–	–
				$\sum$ of ester compounds		12.82	2.29	3.64

[a] Identification method (fit/retrofit values, %); compound name[(a)]—identified for the first time.
[b] Relative area in percentage.
[c] Tentatively identified by retention indices on HP-5 capillary column.
[d] Tentatively identified by NIST05.
[e] Identified by comparison with reference compound.

Table 3
Identification or tentative identification of terpens in *C. roseus*.

Number	Retention time/RI values	Quantification ions	EIMS fragmentation (relative intensity, %)	Name	ID[a] (fit/Rfit)	RA[b] (%) leaves	RA (%) stems	RA (%) flowers
42	10.03/976	69; 93	93 (100); 69 (52); 91 (47); 79 (32); 77 (26); 67 (23); 121 (19); 92 (16); 80 (14); 53 (13)	β-Pinene[(a)]	RI[c], MS[d]	0.30	4.95	0.55
43	11.24	67; 93	68 (100); 67 (87); 93 (51); 39 (37); 41 (36); 79 (32); 53 (31); 27 (24); 94 (23); 91 (19)	Limonene	S[e], MS	–	0.68	37.98
44	11.36	108; 139	67 (100); 95 (48); 93 (32); 81 (28); 96 (23); 65 (22); 66 (16); 109 (15); 55 (15); 112 (14)	1,8-Cineol	MS (84,0/87,2)	–	1.03	–
45	13.35	93; 121	68 (100); 67 (87); 93 (51); 39 (37); 41 (36); 79 (32); 53 (31); 27 (24); 94 (23); 91 (19)	Linalool	S[e], MS	–	–	0.24
46	14.58	79; 93; 108	79 (100); 67 (99); 93 (73); 94 (73); 81 (54); 108 (40); 55 (33); 95 (31); 77 (31); 91 (31)	Limonene oxide[(a)]	MS (75,4/84,1)	–	–	0.35
47	14.89	81; 95; 108	95 (100); 81 (77); 67 (41); 108 (37); 93 (37); 55 (30); 109 (26); 79 (23); 83 (22); 69 (20)	Camphor[(a)]	MS (73,2/81,2)	–	–	0.15
48	15.12	77; 103; 139	103 (100); 104 (92); 77 (90); 112 (54); 139 (49); 97 (49); 55 (46); 69 (45); 84 (42); 132 (32)	*cis*-Menthone[(a)]	MS (51,5/74,7)	–	–	0.19
49	15.74	81; 95; 123	71 (100); 81 (89); 95 (80); 67 (41); 55 (39); 82 (38); 41 (36); 123 (36); 69 (33); 96 (28)	Menthol[(a)]	S[e], MS	–	–	1.26
50	16.26	93; 121; 136	93 (100); 59 (89); 121 (69); 57 (64); 81 (56); 67 (47); 71 (47);92 (41), 136 (40); 79 (33)	(+)-α-Terpineol[(a)]	S[e], MS	–	–	0.51
51	17.64	82; 93; 108	82 (100); 54 (46); 39 (32); 93 (31); 108 (26); 53 (20); 107 (19); 41 (18); 79 (17); 91 (15)	(+)-Carvone[(a)]	MS (74,9/85,4)	–	–	0.62
52	18.53	81; 95; 131	71 (100); 81 (89); 95 (80); 67 (41); 55 (39); 82 (38); 41 (36); 123 (36); 69 (33); 96 (28)	L-(−)-Menthol[(a)]	S[e], MS	–	–	0.23
53	22.98	107; 151	43 (100); 41 (39); 69 (25); 67 (7); 107 (7); 53 60 151 (5); 93 (5); 136 (5); 55 (4)	*trans*-Geranylcetone	S[e], MS	3,87	–	1.26
54	28.69	69; 109	119 (100); 109 (67); 67 (63); 69 (54); 93 (53); 95 (38); 79 (31); 55 (30); 105 (27); 71 (27)	α-Bisabolol[(a)]	S[e], MS	–	–	0.38
55	35.11	275; 257	275 (100); 257 (74); 81 (51); 192 (49); 55 (47); 137 (43); 177 (39); 95 (39); 67 (36); 43 (35)	Manoyl oxide[f,(a)]	MS (78,5/80,8)	–	–	–
56	35.52	257; 81	257 (100); 67 (55); 81 (39); 95 (35); 55 (34); 79 (27); 109 (26); 275 (25); 107 (24); 191 (23)	epi-Manoyl oxide[f,(a)]	MS (78,9/80,9)	–	–	–
57	36.18	81; 107	81 (100); 95 (86); 79 (63); 71 (54); 93 (52); 55 (46); 91 (44); 105 (43); 67 (41); 107 (40)	Manool[f,(a)]	S[e], MS	–	–	–
58	37.09	71; 81; 95; 123	71 (100); 81 (60); 95 (44); 123 (36); 55 (28); 57 (26); 69 (25); 97 (23); 83 (22); 67 (22)	*trans*-Phytol (1)	S[e], MS	3.67	–	–
				∑ of terpene compounds		7.85	6.67	43.72

[a] Identification method (fit/retrofit values, %); compound name[(a)]—identified for the first time.
[b] Relative area in percentage.
[c] Tentatively identified by retention indices on HP-5 capillary column.
[d] Tentatively identified by NIST05.
[e] Identified by comparison with reference compound.
[f] Determined only in the lyophilized extracts.

Table 4
Identification or tentative identification of carotenoid derivatives in *C. roseus*.

Number	Retention time/RI values	Quantification ions	EIMS fragmentation (relative intensity, %)	Name	ID[a] (fit/Rfit)	RA[b] (%) leaves	RA (%) stems	RA (%) flowers
59	14.99/1120	70; 81	70 (100); 69 (83); 67 (48); 79 (22); 81 (19); 53 (18); 94 (17); 55 (16); 77 (16); 91 (15)	*cis*, *trans*-2,6-Nonadienal[(a)]	S[c], RI[d], MS[e]	0.25	0.27	0.16
60	16.41/1202	121; 105	91 (100); 107 (74); 121 (59); 105 (52); 79 (39); 77 (26); 55 (22); 150 (21); 65 (13); 93 (13)	Safranal	RI, MS	0.12	–	–
61	16.85	81; 88	81 (100); 73 (23); 67 (21); 281 (19); 79 (12); 82 (10); 55 (10); 95 (9); 53 (9); 88 (9)	*trans*, *cis*-2,4-Decadienal	S[c], MS	–	1.46	–
62	16.99	123; 152	67 (100); 109 (87); 81 (77); 123 (74); 137 (67); 152 (58); 91 (49); 79 (46); 95 (42); 77 (33)	β-Cyclocitral	S[c], MS	1.12	–	–
63	18.00	107; 151	95 (100); 107 (91); 81 (82); 151 (78); 91 (55); 133 (52) 79 (49); 67 (42); 82 (40); 105 (32)	β-Homocyclocitral	S[c], MS	0.38	–	–
64	18.27	69; 97; 137	69 (100); 97 (90); 84 (37); 67 (29); 94 (27); 137 (24); 83 (23); 79 (19); 81 (17); 65 (16)	Cytral[(a)]	MS (65,9/89,3)	–	–	0.23
65	19.03	81	81 (100); 67 (22); 83 (16); 95 (16); 79 (15); 55 (14); 53 (11); 65 (10); 77 (10); 66 (8)	*trans*, *trans*-2,4-Decadienal[(a)]	S[c], MS	–	0.75	–
66	22.42	93; 121; 177	121 (100); 93 (70); 91 (45); 77 (32); 136 (17); 92 (17); 109 (14); 177 (13); 192 (12); 79 (12)	α-Ionone	RI, MS	0.27	2.21	0.25
67	22.69	121; 161; 176	121 (100); 93 (79); 161 (67); 79 (51); 91 (45); 81 (41); 105 (40); 119 (40); 176 (36); 95 (35)	Dihydro-β-ionone[(a)]	MS (84,1/85,9)	–	1.46	–
68	23.86	177	177 (100); 91 (22); 178 (16); 79 (15); 93 (15); 77 (14); 135 (12); 107 (12); 95 (12); 105 (11)	β-Ionone	S[c], MS	18.22	0.55	0.40
69	23.94	123; 135	123 (100); 135 (11); 95 (9); 124 (8); 79 (7); 91 (6); 107 (6); 77 (5); 67 (5); 109 (5)	2,3-Epoxy-α-ionone	MS (88,6/89,1)	7.83	0.19	0.14
70	25.21/1561	111; 137; 181	111 (100); 109 (86); 137 (77); 67 (61); 181 (60); 95 (28); 79 (22); 180 (21); 110 (21); 81 (20)	Dihydroactinidiolide	RI, MS	6.75	0.20	0.09
				$\sum$ of carotenoid derivative compounds		34.95	7.09	1.27

[a] Identification method (fit/retrofit values, %); compound name[(a)]—identified for the first time.
[b] Relative area in percentage.
[c] Identified by comparison with reference compound.
[d] Tentatively identified by retention indices on HP-5 capillary column.
[e] Tentatively identified by NIST05.

constant flow. The oven temperature was 40 °C (for 1 min), then increased at 2 °C/min to 220 °C and held for 30 min. All mass spectra were acquired in the electron impact (EI) mode. Ionization was maintained off during the first 4 min, to avoid solvent overloading. The ion trap detector was set as follows: the transfer line, manifold and trap temperatures were respectively 280, 50 and 180 °C. The mass range was 50 to 600 *m*/*z*, with a scan rate of 6 scan/s. The emission current was 50 μA, and the electron multiplier was set in relative mode to autotune procedure. The maximum ionization time was 25,000 μs, with an ionization storage level of 35 *m*/*z*. The injection volume for liquid extracts was 1 μL and the analysis was performed in FullScan mode. Peaks' areas were determined by re-constructed FullScan chromatogram using for each compound some specific ions (quantification ions, see Tables 1–6). By this way some peaks which were co-eluting in FullScan mode (resolution value lower than 1) were able to be integrated with resolution value higher than 1.

For SPME analysis the oven temperature conditions were the same. The GC injector liner was a SPME specific one and injection was done in splitless mode. Ionization was kept off for only 1 min. The mass range was 35–350 *m*/*z* [11].

Identification was achieved by comparisons of mass spectra obtained from the sample, with those from pure standards injected in the same conditions; by comparing the Kovats indices and the mass spectra present in the NIST 05 MS Library Database or in the literature.

Table 5
Identification or tentative identification of nitrogen containing compounds, *p*-hydroxycinnamic acid and volatile phenols in *C. roseus*.

Number	Retention time/RI values	Quantification ions	EIMS fragmentation (relative intensity, %)	Name	ID[a] (fit/Rfit)	RA[b] (%) leaves	RA (%) stems	RA (%) flowers
71	5.26/820	66; 93	93 (100); 66 (58); 92 (26); 65 (23); 67 (20); 51 (16); 78 (16); 94 (10); 52 (9); 50 (7)	2-Methyl-pyridine[(a)]	RI[c], MS[d]	0.07	0.05	–
72	6.91/887	66; 107	107 (100); 106 (45); 66 (41); 65 (23); 92 (20); 79 (18); 77 (11); 108 (9); 63 (8); 80 (6)	2,6-Dimethylpyridine[(a)]	RI, MS	0.07	0.04	–
73	8.24/941	79; 107	107 (100); 106 (59); 79 (51); 92 (24); 65 (22); 80 (15); 77 (15); 108 (10); 66 (9); 51 (8)	2,4-Dimethylpyridine[(a)]	RI, MS	0.20	–	–
74	9.08/959	92; 106	92 (100); 107 (66); 106 (62); 65 (44); 79 (22); 51 (16); 77 (15); 78 (11); 83 (10); 50 (10)	3-Ethylpyridine[(a)]	RI, MS	0.82	1.07	–
75	11.86	109; 138	138 (100); 109 (70); 120 (60); 107 (55); 137 (52); 82 (51); 57 (44); 54 (42); 55 (35); 68 (35)	3-Methoxy-2,5-dimethylpyrazine[(a)]	MS (72,7/82,5)	–	2.08	–
76	13.04	124; 137; 152	137 (100); 124 (36); 81 (26); 152 (24); 109 (23); 95 (21); 55 (19); 57 (19); 69 (17); 56 (15)	2-Isopropyl-3-methoxypyrazine[(a)]	S[e], MS	0.19	0.75	0.30
77	15.64	124; 151	124 (100); 81 (38); 95 (30); 94 (29); 93 (24); 151 (19); 67 (13); 79 (12); 123 (10); 53 (10)	2-Isobutyl-3-methoxypyrazine[(a)]	S[e], MS	0.38	6.42	0.34
78	17.25/1226	108; 135	135 (100); 108 (39); 69 (27); 82 (16); 91 (15); 55 (10); 136 (10); 63 (9); 81 (8); 58 (7)	1,3-Benzothiazole[(a)]	RI, MS	0.98	1.21	0.73
79	19.08/1276	117; 90	117 (100); 90 (70); 89 (57); 57 (27); 69 (16); 71 (14); 63 (14); 55 (13); 85 (10); 91 (10)	1H-indole[(a)]	RI, MS	0.66	–	0.34
80	20.37/1317	92; 119; 152	119 (100); 92 (76); 151 (53); 65 (34); 120 (27); 63 (11); 64 (11); 93 (9); 95 (9); 9 (8)	Methyl anthranilate[(a)]	S[e], RI, MS	0.49	0.12	0.03
				$\sum$ of all N-compounds		3.85	11.74	1.73
				Acids				
81	38.00	178		*p*-Hydoxycinnamic acid[(a)]	MS[d] (63,5/75,3)	–	–	0.08
				Phenols				
82	11.31	124	124 (100); 123 (49); 95 (11); 125 (8); 39 (6); 107 (6); 69 (6); 67 (5); 55 (5); 77 (5)	5-Methyl-resorcinol[(a)]	MS (70,4/72,0)	0.24	–	–
83	19.50/1312	135; 150	135 (100); 150 (94); 77 (72); 107 (59); 79 (41); 51 (12); 55 (12); 78 (11); 53 (11); 63 (10)	2-Methoxy-4-vinylphenol[(a)]	RI[c], MS	0.54	–	0.14
84	20.60	134; 164	134 (100); 91 (81); 164 (68); 79 (67); 119 (60); 77 (58); 69 (52); 107 (50); 103 (45); 131 (40)	Eugenol	S[e]; MS	0.32	0.17	0.65
				$\sum$ of phenol compounds		1.09	0.17	0.87

[a] Identification method (fit/retrofit values, %); compound name[(a)]—identified for the first time.
[b] Relative area in percentage.
[c] Tentatively identified by retention indices on HP-5 capillary column.
[d] Tentatively identified by NIST05.
[e] Identified by comparison with reference compound.

2.6. Statistical analysis

Principal component analysis (PCA) and agglomerative hierarchic cluster analysis (dendogram) (CAH) were carried out using XLSTAT 2007.5. PCA method shows similarities between samples projected on a plane and makes it possible to find which variables determine these similarities and in what way. The dendogram method shows correlations by clusters diagrams.

3. Results and discussion

Previous work on the volatile composition of *C. roseus* used hydrodistillation extraction with a Clavenger type apparatus [7,8]. As referred above, this technique has the inconvenient of involving high temperature which modifies the real volatile composition of the biological material.

In this study, two different extraction techniques were used for analyzing volatile, semi-volatile and non-volatile compounds in *C. roseus* plant material: extraction with organic solvent (dichloromethane) and HS-SPME (applied to the fresh plant and to the aqueous lyophilized extract). Selection of the most appropriate SPME fibre depends on the compounds targeted and therefore on the plant material under study. The most effective fibres for HS-SPME were those characterized by two components: a liquid (PDMS) for the less polar compounds and a solid (DVB, CAR, or both) polymeric coating for the more polar constituents. In this work three fibres were evaluated with the following phases:

Table 6
Relative percentage (%) of non-volatile compounds in *C. roseus* using dichloromethane extraction.

Number	Retention time/RI values	Quantification ions	EIMS fragmentation (relative intensity, %)	Name	ID[a] (fit/Rfit)	RA[b] (%) leaves	RA (%) stems	RA (%) flowers
85	12,55	106; 120; 121	106 (100); 121 (99); 120 (98); 79 (68); 77 (63); 91 (31); 78 (17); 65 (16); 93 (16); 107 (14)	3-Ethyl-5-methylpyridine[(a)]	MS[c] (63,1/80,0)	0.42	–	–
86	23,57	91; 162; 175	161 (100); 203 (49); 175 (40); 91 (30); 218 (29); 163 (27); 147 (20); 162 (19); 105 (19); 77 (19)	2,2,7,7-Tetramethyltriyclo (6.2.1.0(1,6))undec-4-en-3-one	MS (75,6/78,5)	0.82	–	–
87	24,82	93; 107; 135	107 (100); 135 (82); 91 (75); 79 (49); 93 (47); 105 (47); 77 (36); 69 (32); 119 (31); 55 (25)	α-Farnesene[(a)]	MS (71,4/77,8)	0.72	0.08	–
88	31,73	81; 95; 109; 123	107 (100); 135 (82); 91 (75); 79 (49); 93 (47); 105 (47); 77 (36); 69 (32); 119 (31); 55 (25)	3,7,11,15-Tetramethyl-2-hexadecen-1-ol[(a)]	MS (82,8/85,4)	2.97	–	–
89	44,86	135; 170; 230; 336	170 (100); 135 (84); 134 (82); 336 (66); 156 (58); 120 (45); 169 (40); 230 (39); 77 (37); 216 (36)	Alkaloid-like compound 1	MS (84,3/86,4)	44.03	44.03	–
90	45,23	134; 170; 230; 336	170 (100); 134 (78); 135 (72); 336 (67); 156 (42); 120 (41); 154 (40); 230 (39); 169 (35); 216 (35)	Alkaloid-like compound 2	MS (85,9/87,8)	34.91	38.21	36.32
91	45,65	134; 168; 216; 336	336 (100); 207 (94); 57 (83); 69 (71); 134 (70); 135 (65); 67 (63); 170 (63); 91 (62); 55 (57)	Alkaloid-like compound 3	MS (74,0/80,9)	0.40	1.36	3.16
92	45,91	135; 156; 216; 336	135 (100); 156 (81); 216 (70); 336 (67); 107 (58); 229 (45); 79 (35); 77 (34); 134 (32); 154 (32)	Alkaloid-like compound 4	MS (76,0/78,6)	1.59	3.28	–
93	47,86	136; 180; 263; 322; 338	338 (100); 136 (87); 180 (78); 263 (71); 322 (68); 154 (63); 323 (45); 124 (34); 167 (34); 232 (33)	Alkaloid-like compound 5	MS (67,9/73,2)	1.84	5.71	27.8
94	50,01	108; 167; 194; 227; 334	167 (100); 194 (91); 227 (83); 168 (82); 108 (81); 334 (61); 166 (42); 195 (39) 226 (22); 182 (22)	Alkaloid-like compound 6	MS (52,2/52,8)	5.15	5.65	–
95	51,63	180; 263; 322; 350	322 (100); 321 (99); 169 (92); 168 (89); 263 (35); 307 (33); 249 (27); 154 (21); 323 (21); 115 (19)	Alkaloid-like compound 7	MS (65,0/70,8)	0.46	–	–
96	54,58	261; 307; 321; 366	321 (100); 57 (49); 307 (35); 261 (35); 71 (34); 85 (31); 366 (28); 320 (25); 322 (22); 181 (17)	Alkaloid-like compound 8	MS (61,3/64,6)	1.77	1.68	–
97	56,38	169; 263; 307; 322	322 (100); 169 (97); 321 (77); 168 (72); 307 (35); 263 (34); 207 (29); 249 (25); 93 (22); 115 (20)	Alkaloid-like compound 9	MS (61,8/66,8)	1.02	–	–
98	70,40	156; 184; 325	207 (100); 352 (67); 351 (60); 156 (56); 209 (41); 281 (39); 184 (32); 73 (31); 129 (25); 55 (21)	Alkaloid-like compound 10	MS (64,6/69,1)	–	–	69.8

[a] Identification method (fit/retrofit values, %);
[b] Relative area in percentage.
[c] Tentatively identified by NIST05.
[(a)] compound name—identified for the first time.

CAR/PDMS, CW/DVB and DVB/PDMS. The last one has the particularity to have a specific selectivity to nitrogen containing compounds.

3.1. Comparison of SPME fibres

Using the CAR/PDMS fibre 25 compounds were identified: n-hexanal, 2-hexen-1-ol, *cis*-3-hexen-1-ol, hexanol, benzaldehyde, 1,4-cineole, limonene, benzylic alcohol, eucalyptol, 3-methoxy-2,5-dimethylpyrazine, phenylacetaldehyde, linalool, 2-nonen-1-ol, phenylethanol, camphor, isobutylmethoxypyrazine, *trans*-2-decenol, verbenone, β-cyclocitral, bornilacetate, eugenol, valencene, β-ionone, epoxy-β-ionone and *trans*-geranylacetone. When CW/DVB fibre was applied, some diterpenic compounds, as manool and their oxides, and α-bisabolol were detected, in addition to the above mentioned compounds. Finally, using the DVB/PDMS, 10 aldehydes, 14 alcohols, 8 esters, 10 nitrogen compounds, 12 terpenes, 8 carotenoid derivatives, 4 ketones plus 3 other compounds

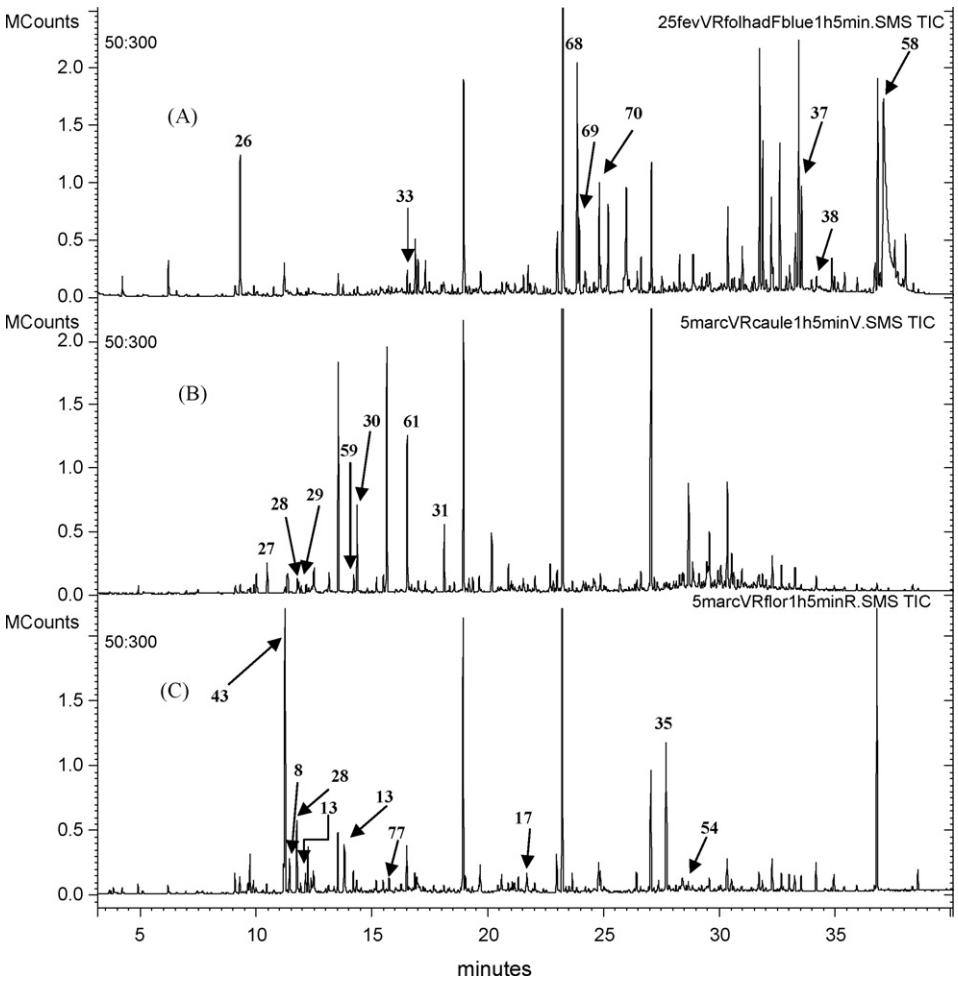

Fig. 1. Chromatograms of the SPME using DVB; PDMS fibre analysis in leaves (A), stems (B) and flowers (C) of *C. roseus*. The corresponding compound names are shown in Tables 1–6. Chromatographic conditions: oven temperature −40 °C (for 1 min), 2 °C/min to 220 °C and held for 30 min. Injector port was heated to 220 °C, in splitless mode. Carrier gas—helium C-60 (Gasin, Portugal), at 1 mL/min, constant flow. Chromatographic column—VF-5ms 30 m × 0.25 mm × 0.25 μm (FactorFour) from VARIAN.

were identified for the first time in fresh flowers, stems and leaves of *C. roseus*.

3.2. Identification and semi-quantification of volatile molecules extracted by SPME

SPME allowed the determination of 12 aldehydes, 14 alcohols, 9 esters, 10 nitrogen containing compounds, 17 terpenic compounds (including aliphatic mono and diterpenes), 12 carotenoid derivatives, 6 ketones, 1 hydoxycinnamic acid and 3 phenol compounds, as it is listed in Tables 1–5. Among the identified compounds, only compounds numbered as **16**, **20**, **26**, **27**, **29**, **30**, **31**, **37**, **43**–**45**, **53**, **58**, **60**–**62**, **66**, **68**, **70** and **84** (Tables 1–5), were previously described in *C. roseus* [7,8]. Some of them are present in flowers, leaves and stems, while others exist only in a certain organ of the plant as it is shown in Fig. 1.

Flowers were richer in phenylacetaldehyde and in the correspondent alcohol, 2-phenylethanol, than the other plant organs (Tables 1 and 2). 2-Phenylethanol is responsible for the rose-note aroma. These two molecules have an important biological function in plants. The latter has long been known to possess antimicrobial properties [14] and its synthesis by plant reproductive structures may indicate a protective role for flowers and fruits. Both 2-phenylacetaldehyde and 2-phenylethanol are also potent insect attractants [15,16].

Flowers also exhibited high amounts of mono- and diterpenic compounds. These molecules have been found before only in the essential oil of the leaves of *C. roseus* [7]. Among monoterpenes, it can be highlighted their high limonene amounts (Table 3). Monoterpenes are, among the most volatile compounds, those with more pleasant aroma descriptors.

Diterpenic compounds, such as α-bisabolol and manool and their oxide compounds were found in low levels in the headspace of flowers and leaves. Their presence is well noticed in lyophilized extracts of leaves using the DVB/PDMS fibre (Fig. 2). A number of biological activities have been described for abietane diterpenoids, namely cardiovascular, antiulcer, antioxidant, antitumor, tuberculostatic and antiviral activities. It has also been described that these molecules possess antimicrobial activity [17,18]. Manoyl oxide and epi-manoyl oxide belong to labdane diterpenes. Labdanes is a group of natural products isolated from several plant families with a wide range of biological activities [16]. Manoyl oxide and most of the labdane diterpenes with unsaturated side chain are present as mixtures of C13 epimers [19,20].

Bisabolol, or more formally α-(−)-bisabolol, is a natural monocyclic sesquiterpene alcohol which was found on *C. roseus* flowers (Table 3). It is a constituent of the essential oil from German chamomile (*Matricaria recutita*) and *Myoporum grassifolium*. It has a weak sweet floral aroma and is used in various fragrances. It has

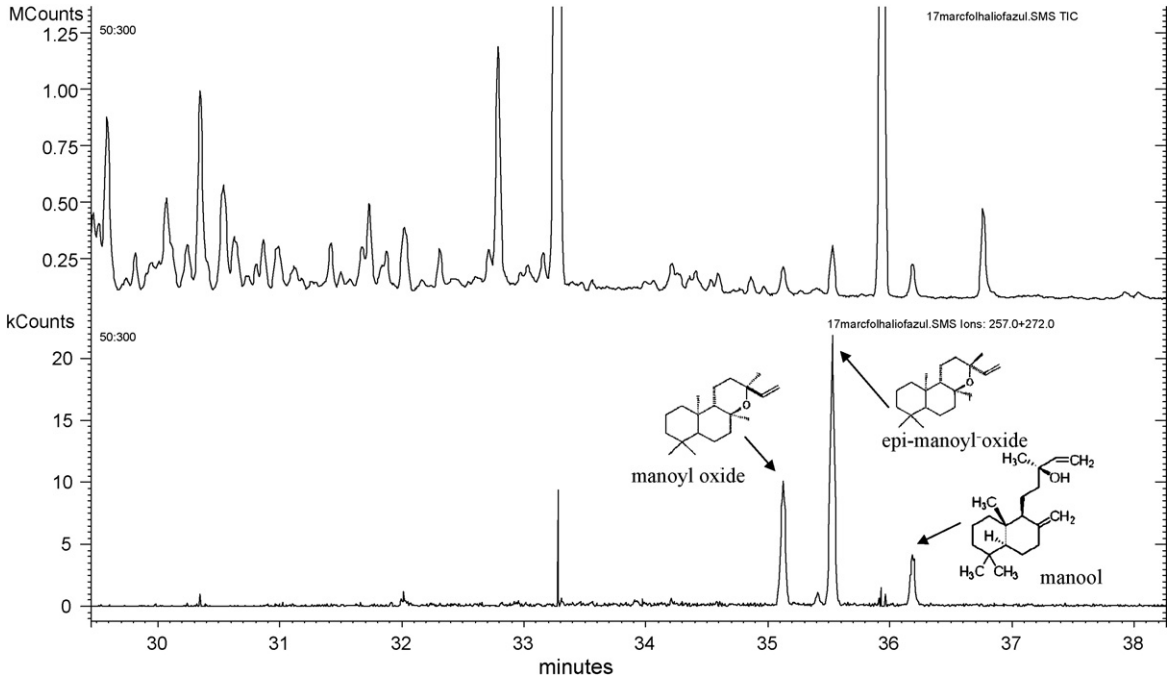

Fig. 2. Chromatogram of the HS-SPME lyophilized extract of leaves. Upper chromatogram in FullScan acquisition, down chromatogram by selected ion monitoring (m/z=257; m/z=272). Chromatographic conditions: oven temperature −40 °C (for 1 min), 2 °C/min to 220 °C and held for 30 min. Injector port was heated to 220 °C, in splitless mode. Carrier gas—helium C-60 (Gasin, Portugal), at 1 mL/min, constant flow. Chromatographic column—VF-5ms 30 m × 0.25 mm × 0.25 μm (FactorFour) from VARIAN.

also been used for hundreds of years in cosmetics because of its perceived skin healing properties, also presenting anti-bacterial and anti-fungal activities [21].

C. roseus flowers are also rich in methyl jasmonate (Table 2). Jasmonates are a group of plant stress hormones [22]. Upon exposure to stress (e.g., wounding and pathogens), jasmonates are produced in plants and cause the induction of a proteinase inhibitor [23]. A coordinated activation of programmed cell death and defense mechanisms often accompany the antimicrobial response of plants [24]. In addition, jasmonates can suppress the proliferation of human cancer cells and induce their death. Methyl jasmonate induced death in breast and prostate carcinoma cells, as well as in melanoma, lymphoma, and leukemia cells [25,26]. It is a chemical inducer of secondary metabolism and it was demonstrated that methyl jasmonate increased the activity of tabersonine epoxidase in hair root cultures of *C. roseus* [27]. Jasmone is produced within plants by jasmonate metabolism, from linolenic acid, by the octadecanoid pathway. It can act as either an attractant or a repellent for various insects [26].

The volatile profile of leaves also comprises different classes of compounds. Among aldehydes and alcohols, high amounts of benzaldehyde and *cis*-hex-3-en-1-ol, could be noted, respectively (Tables 1 and 2). Leaves also present high levels of carotenoid derivatives, such as β-ionone, 2,3-epoxy-β-ionone, β-cyclocitral and dihydroactinilidiolide (Table 4). All these molecules are degradation products of carotenoids, such as carotene and lutein [28]. β-Ionone and β-cyclocitral are known to be important contributors to the flavor aroma of several fruits and wines [28–30]. Additionally, leaves contained important amounts of esters compounds (12.8%), including isopropyl and methyl esters of fatty acids, di- and trisaturated (Table 2). These results are in agreement with those of Pandey-Rai et al. [8] who found in the essential oil of *C. roseus* leaves 18.2% of fatty acids and their esters. Brun et al. [7] have mainly found in the essential oil of leaves 84.8% of fatty acids and esters.

Leaves presented 4.9% of hexahydrofarnesylacetone (Table 1), which is similar to the results obtained by Brun et al. [7] with their essential oil (4%). Recent studies in essential oil of *Scutellaria barbata* have shown that this molecule has an antimicrobial activity [31]. Among terpenic compounds, leaves are richer in *trans*-geranylcetone and *trans*-phytol (Table 3). Higher quantity of this last molecule can be found only in this matrix, which is in agreement with previous results [6]. Phytol is a natural linear diterpene alcohol which is involved in the synthesis of vitamins E and K_1. It is also a decomposition product of chlorophyll [32]. γ-Decalactone is another compound present in high amounts in the headspace of leaves (Table 1).

A great variety of volatile nitrogen containing compounds was also found in leaves, namely pyridine and pyrazine, and thiazole compounds (Table 5). *C. roseus* is a plant known for the presence of important alkaloids with recognized health value, namely anticancer activity [1]. These alkaloids are nitrogen compounds with L-tryptophane as precursor, with a known biosynthetic pathway.

No data could be found in the literature concerning volatiles of *C. roseus* stems. Stems showed higher levels in particular compounds, namely 2-isobutyl-3-methoxypyrazine. Pyrazine compounds are heterocyclic nitrogen containing compounds with unique organoleptic properties. Methoxy pyrazines (MP) are very potent odorants and have a distinctive smell, similar to freshly cut green bell pepper or green peas. The human olfactory thresholds for MP are extremely low, in the range of 2 ng/L in water [33]. Some recent works attribute to pyrazines some antimicrobiological properties [34]. In addition, 2-(allylthio)pyrazine, a cancer chemopreventive agent, inhibits liver fibrosis induced by dimethylnitrosamines [35]. This specific pyrazine was not identified in the analyzed matrices.

The presence of high amounts of *trans*-2-decen-1-ol and 2-none-1-ol, must be referred (Table 2). This last compound has been shown to act as repellent of some insects [36].

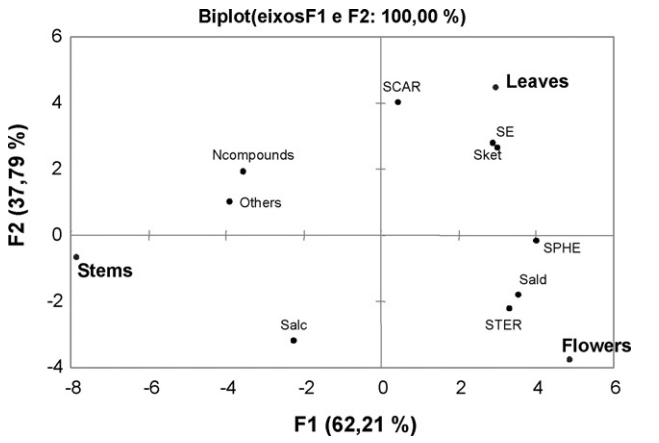

Fig. 3. Principal component analysis of all volatiles compounds analyzed by HS-SPME–GC–MS grouped by family classes in flowers, stems and leaves. SCAR—sum of carotenoid molecules, Sket—sum of ketones, SPHE—sum of phenols, SE—sum of esters compounds, Salc—sum of alcohols, Sald—sum of aldehydes, STER—sum of terpenes, and Ncompounds–nitrogen containing compounds.

Considering ketones, particular attention may be focused on the 6-methyl-5-hepten-2-one found in all parts of plant, but with higher contents in stems (Table 1). It has been reported to be an oxidative by-product or degradation product derived from licopene, farnesene, citral or conjugated tri-enols [37,38].

3.3. Identification and semi-quantification of volatile molecules extracted by dichloromethane

Dichloromethane extraction allowed the identification of 14 other compounds (Table 6). Among these compounds some structures like alkaloid molecules could be identified, as well as 2,2,7,7-tetramethyltricyclo(6.2.1.0(1,6))undec-4-en-3-one, α-farnesene, phytol (3,7,11,15-tetramethyl-2-hexadecen-1-ol). 2,2,7,7-Tetramethyltricyclo(6.2.1.0(1,6))undec-4-en-3-one was recently reported [39] as one of the major constituents of the essential oil of *Aristolochia mollissima*, which have been proved to have antimicrobial activity and cytotoxicity against four cancer cell lines (ACHN, Bel-7402, Hep G2 and HeLa).

Stems are richer in alkaloid compounds than leaves and flowers. There is an exception for alkaloid-like compound 10, which was found only in the flower extract.

In order to assemble the different identified compounds according to the organ of the plant (leaves, stems and flowers), a principal component analysis (PCA) was performed, using the results obtained from the HS-SPME analysis. Fig. 3 shows the projection of chemical variables (sum of compounds of each chemical family) into the plans F1 and F2. Three distinct groups have been formed. Succinctly, flowers were richer in terpene molecules (including limonene), aldehyde compounds, esters compounds, namely methyljasmonate, and phenols (due to the high amounts of eugenol) (Table 5). Leaves are well correlated to the carotenoid derivative compounds, sum of ketone and ester compounds. Finally, stems are in good correlation with nitrogen containing compounds, alcohol and miscellaneous compounds. In order to select, among all volatiles, those which could be markers of each organ of the plant, an agglomerative hierarchic cluster analysis (HCA) was performed. By this way it was possible to restrict the volatiles to nine compounds. Leaves can be characterized by their levels in hexanol, benzaldehyde, palmitic acid methyl ester and *trans*-phytol, flowers by their contents in 1-phenylethanol, limonene and other terpenes, and, finally, stems by their *a*-ionone and *trans*-2-decen-1-ol amounts.

4. Conclusions

A deeper knowledge of *C. roseus* volatile constituents was achieved by the use of HS-SPME fibre. SPME proved to be sensitive, reproducible, and cost efficient, becoming a powerful tool when combined with gas chromatography–mass spectrometry (GC–MS) analysis. It incorporates extraction, concentration and sample introduction into a single step. In fact, using the DVB/PDMS fibre 73 compounds were identified for the first time in fresh flowers, stems and leaves of *C. roseus*. Some of these compounds have an important bioactivity role in human body.

Moreover, statistical analysis allowed the distinction of the different organs of the plant (leaves, stems and flowers) in what concerns their volatile composition.

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