Supplementary material

Chemical composition of the leaf oils from two morphotypes of *Psidium cattleyanum* at four phenological stages

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Abstract: The chemical composition of volatile oil extracted from leaves of two morphotypes of *Psidium cattleyanum* Sabine from southern Brazil was evaluated at four phenological stages. In addition, plant material was evaluated at four different locations of the Planalto Serrano Region of Santa Catarina state, Brazil. Major components found were: 1,8-cineole, α-pinene, α-eudesmol, (E)-caryophyllene and *p*-cymene in red morphotype whereas 1,8-cineole, α-pinene, myrcene, (E)-caryophyllene and valerianol were found in the yellow morphotype. The differences observed in the chemical composition in red and yellow morphotypes may help in the differentiation between the two morphotypes in the absence of fruits. Also, important differences were observed mainly for 1,8-cineole and α-pinene percentage at different phenological stages.

1. Experimental

1.1. Plant material

Leaves from both morphotypes of *Psidium cattleyanum* were collected at four different locations of the Planalto Catarinense region of Santa Catarina State. Three places around Lages municipality (EPAGRI - EP; Morro Grande - MG; Pedras Brancas – PB) and one place in Bom Retiro municipality (BR) (see table S1 and figure S1 and S2 for geographical coordinates and edaphic factors). For each location, leaves were collected in different phenological stages named as old leaves/leaf-fall (OL; June and August 2017), young leaves/flower buds (YL; October and November 2017), flowering (FL; November and December 2017) and fruiting (FR; February and march 2017) (Figure S2 and table S1). Plants were randomly selected, and the same individuals were used for all collections.

Table S1 – Geographical coordinates and altitude for local of collection.

Location	Geographical coordinates	Altitude (m)	Soil Classification
EPAGRI (EP)	27º49’53,13’’S; 50^{0}19’31,9’’W	945	The Cambisols (WRB/FAO) are acid with low rate of base saturation and high values of Al^{3+}.
Morro Grande (MG)	27º49’53,2’’S; 50º 9’32,6’’W	912	
Pedras Brancas (PB)	27º51’53,0’’S; 50º12’51,0’’W	903	
Bom Retiro (BR)	27º50’02,7’’S; 49º32’04,8’’W	832	

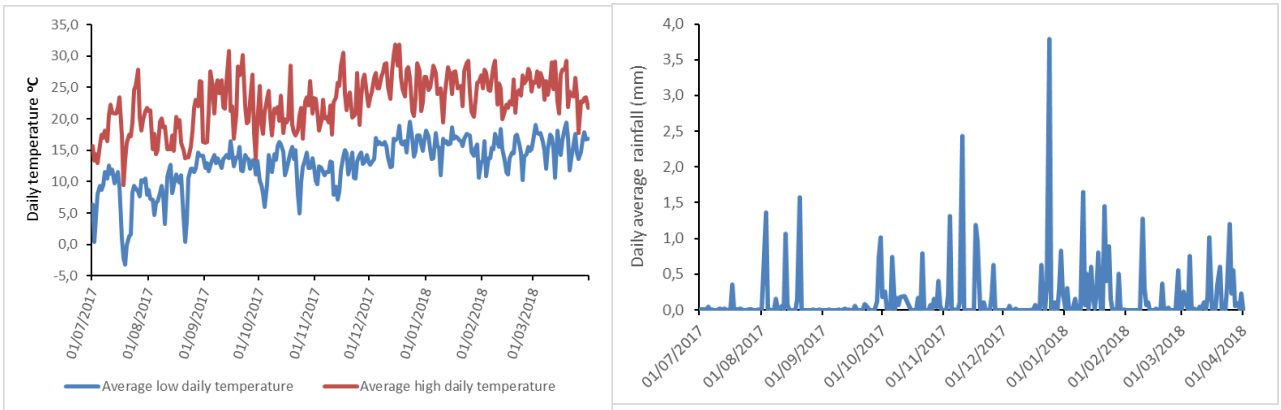

Figure S1 – Data for average daily temperature (left) and daily average rainfall (right) for period when leaves were collected and submitted to hydrodistillation. *Source: INMET-1583 station – Lages, Santa Catarina, Brazil.*

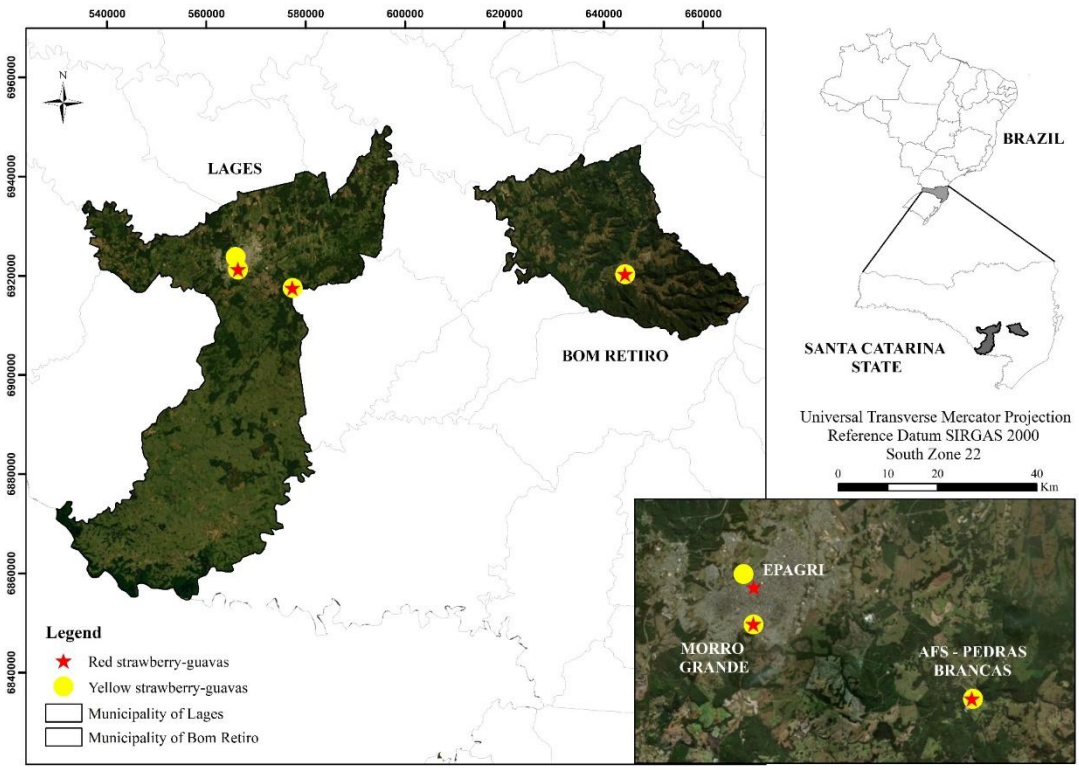

Figure S2 - Map with the sites where the samples of leaves of *Psidium cattleyanum* were collected. *Source: Prepared by the authors, 2019*

Figure S3 – Pictures of phenological stages for red morphotype of *Psidium cattleyanum*. A) Old leaves (OL); B) Young leaves (YL); C) Flowering (FL); D) Fruiting (FR). *Pictures taken by the authors at Pedras Brancas, Lages, SC, Brazil, 2017.*

Table S2 – Schedule for the collection of leaves of *Psidium cattleyanum*.

Phenological stage	Morphotype	Collection date	Location
Old leaves (OL)	Yellow	28/07/17	Epagri (EP)
Old leaves (OL)	Red	31/07/17	Epagri (EP)
Old leaves (OL)	Yellow	02/08/17	Morro Grande (MG)
Old leaves (OL)	Red	08/08/17	Morro Grande (MG)
Old leaves (OL)	Yellow	09/08/17	Pedras Brancas (PB)
Old leaves (OL)	Red	09/08/17	Pedras Brancas (PB)
Old leaves (OL)	Yellow	20/08/17	Bom Retiro (BR)
Old leaves (OL)	Red	20/08/17	Bom Retiro (BR)
Young leaves (YL)	Red	23/10/17	Epagri (EP)
Young leaves (YL)	Yellow	24/10/17	Epagri (EP)
Young leaves (YL)	Red	26/10/17	Morro Grande (MG)
Young leaves (YL)	Yellow	27/10/17	Morro Grande (MG)
Young leaves (YL)	Red	05/11/17	Bom Retiro (BR)
Young leaves (YL)	Yellow	05/11/17	Bom Retiro (BR)
Young leaves (YL)	Yellow	12/11/17	Pedras Brancas (PB)
Young leaves (YL)	Red	12/11/17	Pedras Brancas (PB)
Flowering (FL)	Red	27/11/17	Morro Grande (MG)
Flowering (FL)	Yellow	05/12/17	Morro Grande (MG)
Flowering (FL)	Red	03/12/17	Bom Retiro (BR)
Flowering (FL)	Yellow	03/12/17	Bom Retiro (BR)
Flowering (FL)	Yellow	07/12/17	Epagri (EP)
Flowering (FL)	Red	11/12/17	Epagri (EP)
Flowering (FL)	Yellow	11/12/17	Pedras Brancas (PB)
Flowering (FL)	Red	11/12/17	Pedras Brancas (PB)
Fruiting	Red	06/02/18	Morro Grande (MG)
Fruiting	Yellow	08/02/18	Morro Grande (MG)
Fruiting	Red	15/02/18	Epagri (EP)
Fruiting	Yellow	01/03/18	Epagri (EP)
Fruiting	Red	18/02/18	Bom Retiro (BR)
Fruiting	Yellow	18/02/18	Bom Retiro (BR)
Fruiting	Red	08/03/18	Pedras Brancas (PB)
Fruiting	Yellow	08/03/18	Pedras Brancas (PB)

1.2. Isolation of the essential oil

Essential oils were obtained by hydrodistillation using a 5 liter Clevenger type apparatus. Leaves (230 – 300 g) were selected and washed with water, dried and grounded in small pieces (3 cm). The material was submitted to the extraction in water at boiling temperature for 2 hours. The oil was separated and dried over anhydrous Na_2SO_4, filtered and kept in a sealed glass at -22 ^{0}C.

1.3. Gas chromatography-mass spectrometry (GC-MS) analysis

Analysis of the chemical constituents were carried out on a Agilent 7890A/5975C gas chromatograph-mass spectrometer (GC-MS) equipped with an Agilent Combi PAL – GC autosampler and a fused silica capillary column (DB-5; 30 m x 0.25 mm x 0.25 μm). Solutions of 20 μL of essential oil sample in 1.0 ml of hexanes were prepared for injection of 1 μL in the GC-MS. For chromatography separation helium was used as carrier gas at flow rate of 1.0 mL min^{-1}. The oven temperature was set from 60 ºC to 280 ºC at rate of 4 ºC min^{-1}. The split ratio was 1:50. The inlet and GC-MS interface temperatures were kept at 300 ^{o}C. The ion source (70 eV) temperature was set at 230 ^{o}C (Total ion chromatograms for essential oil from both morphotypes collected at Bom Retiro site are depicted at figures S3-S10). Peaks were manually integrated using G1701EA GC/MSD Chemstation software. Individual components of the essential oil were identified by comparison of their mass spectra and retention indices, relative to those of a series of *n*-alkanes (C7-C30; Sigma Aldrich), with those reported in literature (see tables S1 and S2) (Adams 2007) . The chemical structure of major compounds 1,8-cineole and α-pinene were confirmed by co-injection of commercially available standards (Sigma-Aldrich).

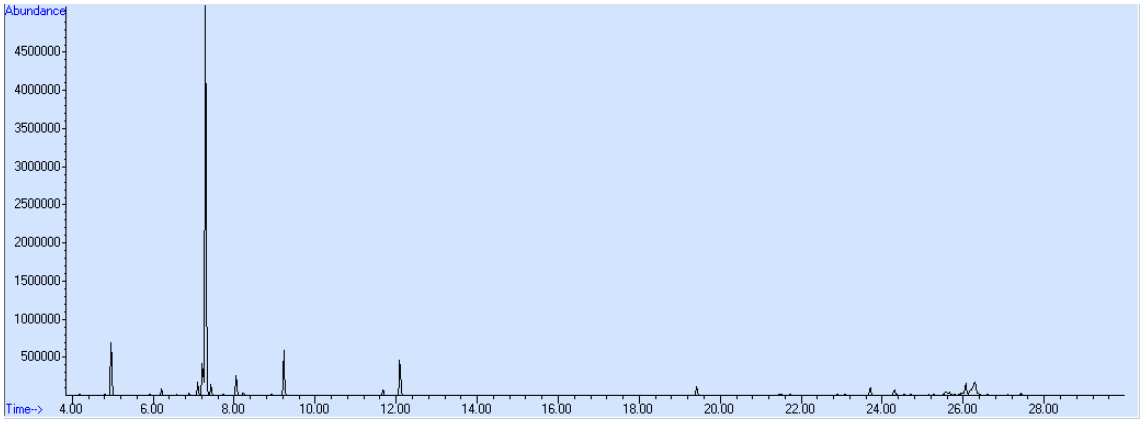

Figure S4 -Total ion chromatogram of the essential oil from the leaves of *Psidium cattleyanum*, red morphotype, collected in Bom Retiro at old leaves stage (BROL).

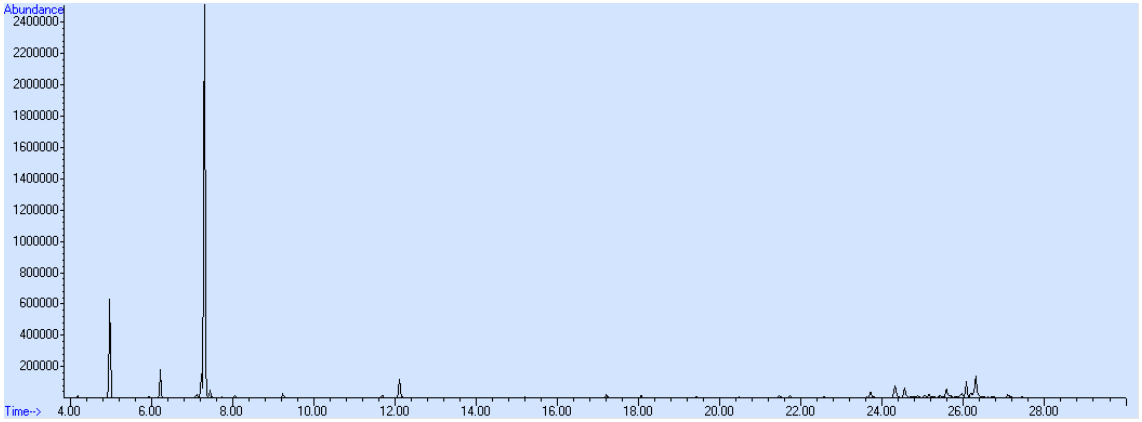

Figure S5 -Total ion chromatogram of the essential oil from the leaves of *Psidium cattleyanum*, yellow morphotype, collected in Bom Retiro at old leaves stage (BROL).

Table S3. Chemical constituents from essential oil isolated from leaves of red morphotype of *Psidium cattleyanum* for different locations.

Peak	Substance	RI exp.	Content (%) / (Local/phenological stage)*														
			EP OL	**EP YL**	**EP FL**	**EP FR**	**MG OL**	**MG YL**	**MG FL**	**MG FR**	**BR OL**	**BR YL**	**BR FL**	**BR FR**	**PB OL**	**PB YL**	**PB FR**
1	α-pinene	949	6.9	18.4	10.2	10.8	3.2	14.5	11.5	8.2	7.3	12.5	3.7	10.6	6.8	15	10.4
2	β-pinene	1003	0.3	0.7	1.8	0.4	0.3	0.7	0.6	0.5	0.4	0.6	0.6	0.4	0.4	0.7	0.6
3	Myrcene	1014	2.6	0.5	1.3	1.6	0.8	0.8	1.8	1.9	1.3	0.6	3.9	1.0	1.4	1.0	1.1
5	α-phellandrene	1028	0.2	0.4	0.3	0.5	0.2	0.6	0.2	0.1	0.2	0.3	1.7	0.2	0.3	0.4	0.5
6	Iso-sylvestrene	1032	0.1	0.2	0.4	0.2	0.05	0.2	1.5	1	0.6	0.1	0.05	0.1	0.1	0.2	0.3
7	α-terpinene	1039	0.4	1.0	0.3	1.0	0	1.6	5.4	2.2	0.3	0.7	0.8	0.4	0.5	0.9	0.9
8	*p*-cymene	1046	0.5	1.3	1.0	2.2	0.8	0.0	19	33.4	0.5	1.6	0.3	2.2	1.0	0.7	3.7
9	Limonene	1050	3.7	3.6	3.4	5.3	2.2	3.3	2.6	2.3	2.8	2.4	5.5	3.3	3.4	3.0	4
10	1,8-cineole	1053	40.4	4.5	24	11.5	55.8	6.8	11.5	0.5	45.2	33.8	11.5	16.3	47.9	20.6	12.8
11	(Z)-β-ocymene	1056	0.8	5.1	0.8	2.6	0.05	4.3	6.3	4.4	0.6	2.5	1	2.8	0.7	3.7	5
12	(E)-β-ocymene	1065	0.9	0.3	0.3	0.4	0.1	0.4	0.4	0.6	0.5	0.4	0.2	0.4	0.4	0.6	0.7
13	σ-terpinene	1076	3.4	4.8	5.1	5.6	2.4	6.2	0.5	0.4	4.1	4	1.9	2.5	4.9	4.9	7.1
14	1,4-pentenyl butyrate	1080	0.1	0.2	0.3	0.1	0.9	0.6	4	4.8	0.7	0.5	0.5	0.4	0.5	0.4	0.4
15	Terpinolene	1100	0.2	0.4	0.5	0.5	0.2	0.6	0.9	0.9	0.4	0.3	0.1	0.3	0.3	0.4	0.6
16	Linalool	1111	4.2	5.8	4.8	2	6.6	6	3	4	5.6	6.5	2.0	4.4	4.7	4.3	4.8
17	4-terpineol	1185	1.3	0.4	0.9	0.8	1.8	0.6	0.8	0.7	1.4	1.1	2.6	0.7	1.7	0.9	1.3
18	α-terpineol	1197	4.1	0.9	3.2	3.0	6.0	1.2	0.5	0.4	5.2	3.5	1.4	3.0	6.0	3.2	3.4
19	E-caryophyllene	1424	0.2	5.8	9.2	10.8	1.5	10.9	1.2	1.2	2.6	2.6	7.6	8.6	1.9	4.8	9.7
20	α-humulene	1458	0.2	0.7	0.8	1.0	0.2	1.0	0.5	0.6	0.2	0.2	0.6	0.8	0.2	0.6	0.9
21	σ-muuroleno	1479	0.2	0.7	0.6	0.6	0.1	1.0	1.8	1.5	0.2	0.3	0.6	0.6	0.4	0.6	1
22	β-selinene	1489	0.2	2.3	1.3	1.4	0.1	2.3	1.4	0.8	0.4	0.8	0.7	1.6	0.2	1.4	2.3

23	α-Selinene	1497	0.2	2.4	1.5	1.7	0.4	2.4	0.9	1.3	0.7	0.9	1.2	1.7	0.4	1.5	2.4
24	Δ- cadinene	1526	0.2	0.8	0.6	0.7	0.1	0.9	1.4	1.3	0.3	0.3	0.5	1.0	0.1	0.5	0.7
25	α-cadinene	1538	0.2	3.4	2.0	2.2	0.2	3.2	1.5	1.02	0.4	1.1	1.6	2.5	0.2	1.4	2
26	3,7 (11)-selinadiene	1545	0.2	3.3	2.0	2.2	0.2	3.2	2.7	2.2	0.4	0.8	1.6	2.4	0.2	1.4	2.2
27	Germacrene B	1560	0.3	5.8	0	0.3	0.1	4.5	4.6	3.8	0.0	1.4	0.7	1.7	0	2.4	1.5
28	(E)-nerolidol	1566	0.3	9.8	5.7	7.6	1.4	6	0.7	0.5	2.4	3.9	3.7	4.7	0.8	3.6	2.7
29	Globulol	1586	0.3	0.7	2.0	2.4	3.5	0.5	0.1	0.1	2.1	2.4	2.1	1.7	2.6	2.4	1.4
30	1-epi-cubenol	1626	0.3	1.3	1.7	2.5	1.6	1.2	0.7	1.4	2.0	1.6	1.7	2.8	1.5	1.9	1.6
31	α-muurolol	1642	0.3	2.1	3.0	3.6	1.5	1.9	2.4	3.6	2.8	2.1	2.4	4.6	2.2	2.4	2.9
32	α-eudesmol	1650	0.3	6.6	7.3	7.7	3.2	4.4	4.6	7.7	4.5	5.6	4.6	8.8	4.8	8.1	6
33	Valerianol	1658	0.3	1.1	0.7	0.9	0.5	4.4	0.6	0.9	0.5	0.7	0.6	1.2	0	0.7	0.7

RI: Kovat's Retention Index determined by the normalisation of retention times with respect to a *n*-alkanes mixture. *EPOL –Epagri, old leaves; EPYL – Epagri, Young leaves; EPFL – Epagri, flowring; EPFR – Epagri, fruiting; MGOL – Morro Grande, old leaves; MGYL – Morro Grande, young leaves; MGFL – Morro Grande, flowering; MGFR – Morro Grande, fruiting; BROL – Bom Retiro, old leaves; BRYL – Bom Retiro, young leaves; BRFL – Bom Retiro, flowering; BRFR – Bom Retiro, fruiting; PBOL – Pedras Brancas, old leaves; PBYL – Pedras Brancas, young leaves; PBFL – Pedras Brancas, flowering.; PBFR - Pedras Brancas, fruiting.

Table S4. Chemical constituents from essential oil isolated from leaves of yellow morphotype of *Psidium cattleyanum* for different locations.

Peak	Substance	RI exp.	Content (%) / (Local/phenological stage)*													
			EP OL	**EP YL**	**EP FL**	**EP FR**	**MG OL**	**MG FR**	**BR OL**	**BR YL**	**BR FL**	**BR FR**	**PB OL**	**PB YL**	**PB FL**	**PB FR**
1	α-pinene	949	15.2	29.1	18.4	9.4	2.8	7.2	12.6	20.0	17.9	11.1	15.0	27.5	31.8	10.4
2	β-pinene	1003	0.7	1.1	0.7	0.4	0.2	0.3	0.32	1	0.5	0.4	1.5	2.6	2.5	0.4
3	Myrcene	1014	13.7	9.8	10.2	10.7	8.9	21.6	4.2	2.7	6.1	4.8	4.7	5.1	7.5	11.6
4	p-cymene	1046	0.4	0.2	0.1	0.1	2.2	0.1	2.3	0.2	0.1	0.1	0.8	0.2	0.2	0.2
5	Limonene	1050	1.5	1.3	1.1	1.7	3.7	2.9	28.3	1.5	1.3	2.1	1.7	1.2	0.9	3.5
6	1,8-cineole	1053	28.1	15.9	5.0	5.3	45.5	25.8	18.4	4.8	16.3	15.1	38.8	7.9	9.9	8.7
7	(Z)-β-ocymene	1056	0.3	1.8	1.0	0.5	0.7	0.9	0.6	6.8	4.7	3.0	0.5	0.4	0.4	1.1
8	σ-terpinene	1076	0.6	0.6	0.6	0.7	1.8	0.9	1.0	0.3	0.5	0.6	1.8	0.4	0.4	0.7
9	Linalool	1111	0.4	0.1	0.1	0.1	4.8	3.9	1.0	1.1	1.2	0.7	4.7	3.1	3.2	2.3
10	4-terpineol	1185	0.4	0.3	0.2	0.3	1.0	0.6	0.7	0.2	0.3	0.3	1.2	0.4	0.4	0.3
11	α-terpineol	1197	1.7	0.8	1.0	1.1	3.2	2.3	3.8	0.8	1.9	1.8	4.2	1.5	1.8	1.1
12	E-caryophyllene	1424	5.9	6.0	14.5	18.1	2.7	10.9	0.4	1.0	2.0	1.0	2.9	9.0	11.9	20.7
13	α- Humulene	1458	0.5	0.6	1.3	1.5	0.2	0.8	0.2	1.9	1.5	1.1	0.4	2.7	2.1	1.9
14	β -selinene	1489	2.2	4.8	6.9	6.9	0.5	1.1	0.9	7.5	5.5	3.2	0.4	3.7	2.0	3.3
15	α- selinene	1497	1.9	4.4	6.5	6.4	0.6	1.4	0.9	7.8	5.9	4.2	0.6	3.3	2.6	3.0
16	Δ-cadinene	1526	0.4	0.8	1.7	2.1	0.2	0.7	0.5	3.5	4.0	3.3	0.3	2.4	1.5	1.8
17	(E)-nerolidol	1566	1.4	1.0	1.1	1.2	1.9	1.0	2.1	6.2	3.8	5.5	1.0	1.9	1.1	1.7
18	Globulol	1586	5.6	2.4	3.1	3.9	4.0	0.0	0.0	1.0	3.6	2.2	1.5	3.3	5.0	3.6
19	1-Epi-cubenol	1626	2.2	1.5	2.1	2.6	2.1	0.0	0.0	1.2	2.5	2.1	1.7	4.4	2.3	2.5
20	α-muurolol	1642	0.8	0.5	0.9	1.0	1.1	0.0	0.0	1.0	0.4	0.7	0.5	0.7	1.1	0.8
21	Valerianol	1658	4.9	5.6	7.2	7.9	2.7	0.0	0.0	3.8	4.6	7.6	4.2	10.1	3.7	7.2

RI: Kovat's Retention Index determined by the normalisation of retention times with respect to a n-alkanes mixture. *EPOL –Epagri, old leaves; EPYL – Epagri, Young leaves; EPFL – Epagri, flowring; EPFR – Epagri, fruiting; MGOL – Morro Grande, old leaves; MGYL – Morro Grande, young leaves; MGFL – Morro Grande, flowering; MGFR – Morro Grande, fruiting; BROL – Bom Retiro, old leaves; BRYL – Bom Retiro, young leaves; BRFL – Bom Retiro, flowering; BRFR – Bom Retiro, fruiting; PBOL – Pedras Brancas, old leaves; PBYL – Pedras Brancas, young leaves; PBFL – Pedras Brancas, flowering.; PBFR - Pedras Brancas, fruiting.