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Scents from Brazilian Cerrado: chemical composition of the essential oil from *Psidium laruotteanum* Cambess (Myrtaceae)#

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ABSTRACT

The chemical composition of the essential oils from the leaves from three different populations of *Psidium laruotteanum* Cambess. (Myrtaceae) from the Brazilian Cerrado were analysed by GC-FID and GC-MS. Forty-five components were identified in the oils. The oil yields were 0.3% (dry weight basis). The oil was rich in monoterpenes and major compounds identified were *p*-cymene (19.4–34.8%), 1,8-cineole (6.9–19.2%) and α-pinene (9.2–11.4%). Although collected in different locations and years, the essential oils composition were quite similar, both qualitative and quantitatively.

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Psidium laruotteanum; Myrtaceae; Cerrado; *p*-cymene; essential oil composition

1. Introduction

Brazilian biodiversity comprises *ca.* one-sixth of the total plant species, and includes different biomes, as the Amazon rainforest, the Atlantic Forest and a savannah area in Central Brazil, called Cerrado (1). Amazon forest is the largest and, by far, the most known Brazilian biome. However, Cerrado, the second largest one, is the most threaten by anthropic pressure. Only a small fraction of the 12,000 known botanical species was chemically investigated, making Cerrado a very promising source for flavour and fragrance applications (2).

Among many species of Myrtaceae, the genus *Psidium* stands out as one of the most common in the Cerrado. In Brazil fifty-nine species are found, forty-four of them endemic, and thirty-one native from the Cerrado area (3).

Psidium laruotteanum Cambess is a 2 m high, wild, small tree, largely distributed in small populations in Central Brazil at Cerrado vegetation (3). It is locally known as 'araçá-cascudo'. The fruit of this little shrub is about the size of an ordinary gooseberry, and the flavour is richer than that of any of the other 'araçás' (4). It serves as food for many species of animals and man. The fruit is of excellent taste, much appreciated for fresh consumption and in the form of jams and jellies, but productivity is low.

Previous studies with *P. laruotteanum* described the content of total phenolic compounds of an infusion from the leaves to be 576.56 mg of gallic acid equivalents per gram of dry extract (5). The authors associated this high phenolic content with a very strong antioxidant activity, evaluated by the DPPH method.

The anti-parasitic activity of extracts (hexane, ethyl acetate and ethanol) from *P. laruotteanum* was evaluated for *Plasmodium falciparum, Trypanosoma cruzi* and *T. brucei gambiense* (6). The ethyl acetate extract from the stems was found to be active against *P. falciparum* (IC_{50}: 16.3 μg/mL), while the hexane and ethyl acetate extracts from the leaves showed activity against *T. brucei gambiense* (IC_{50}: 3.9 μg/mL, and 6.8 μg/mL, respectively). Reference compounds used were chloroquine (IC_{50}: 24 nM), pentamidine (IC_{50}: 4.0 nM) and nifurtimox (IC_{50}: 0.6 μg/mL), respectively. The authors reported no cytotoxic activity against L6 cells for the tested extracts.

The essential oil of *P. laruotteanum* was tested against xylophagus fungi, *Gloeophyllum trabeum* and *Trametes versicolor*. A very small inhibition (ca. 9%) was observed when pure oil was tested, but only against *G. trabeum* (7).

Herein, we report the results of the analysis of the essential oil from the leaves of three different populations of *P. laruotteanum* collected in the Cerrado biome. To the

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Table 1. Plant collection data.

Sample code	Place	Collection date
2410	Altitude: 1137 m Latitude: 15°36′29″S Longitude: 47°44′28″W	10 April 2012
2436	Altitude: 1062 m Latitude: 15°54′00″S Longitude: 47°56′20″W	5 June 2012
2605	Altitude: 1113 m Latitude: 15°57′03″S Longitude: 47°54′46″W	11 June 2013

best of our knowledge, this is the first report on the essential oil composition of this species.

2. Experimental part

2.1. Plant material

Leaves from plants of three different populations (minimum five individuals per population), labelled as 2410, 2436 and 2605, were collected and dried to constant weight in an oven at 38 °C with forced ventilation. All sampling areas were situated in Brasilia, Brazil. Voucher specimens were deposited in the herbarium of Embrapa Genetic Resources and Biotechnology (82,843 and 84,485). Sampling code, place of collection and time of the year are presented in Table 1. According to Brazilian law, collection and access were authorized by the Ministry of Environment (process IBAMA 02001.003166/2013-26).

2.2. Distillation of the essential oil

Dried leaves from a representative sample of each population collected were subjected to hydrodistillation in a modified Clevenger-type apparatus for 2 h. After distillation, the oil was collected, dried with anhydrous sodium sulfate (Na_2SO_4) and stored in a freezer at –8 °C for later analysis.

2.3. Analysis of the essential oil

The essential oil was diluted in dichloromethane in the proportion of 1%, and then 1.0 µL of the solution was injected (split 1:20) into an Agilent 6890 N gas chromatograph equipped with a flame ionization detector (GC-FID) and a HP-5MS fused silica capillary column (Agilent Technologies, 5% phenyl-methylpolysiloxane, 30 m × 0.25 mm × 0.25 µm). Hydrogen was used as carrier gas at a flow rate of 1.0 mL/min. The oven temperature was programmed from 60 to 240 °C at 3 °C/min. Injector temperature was kept at 250 °C and detector temperature at 280 °C. The quantitative data (percentage composition) was obtained by normalization. Samples were injected in triplicate.

Analyses by GC-MS were performed on an Agilent 5973 N mass selective detector coupled to an Agilent 6890 gas chromatograph fitted with a HP-5MS fused silica capillary column (Agilent Technologies, 5% phenyl-methylpolysiloxane, 30 m × 0.25 mm × 0.25 µm). Helium was used as carrier gas at 1.0 mL/min. The mass detector was operated in electronic ionization mode (70 eV), at 3.15 scans/s, with mass range from 40 to 450 u. Transfer line was kept at 260 °C, ion source at 230 °C and analyzer at 150 °C. Oven temperature programme and injection procedure were the same as above.

The components were identified by comparison of their mass spectra with those from the Wiley Registry of Mass Spectral Data (8), as well as by their linear retention indices (LRI), calculated according to Van Den Dool and Kratz (9), after the injection of a homologous series of *n*-alkanes (C_7–C_{26}) in the same conditions as above, and compared to literature data (10).

3. Results and discussion

The essential oil yields (dry weight base) were 0.3 % (2410), 0.4% (2436) and 0.3% (2605). Forty-five compounds were identified in the oils, representing 98.8% (2410 and 2436) and 97.1% (2605) of the total.

A rather similar composition was found for the three oils. They were rich in monoterpenes and oxygenated monoterpenoids, accounting for 90% or more of the oils composition. For all samples, *p*-cymene appeared as the major compound (24.8, 19.4 and 34.0%, respectively, for samples 2410, 2436 and 2605). Consequently, very few sesquiterpenes and their oxygenated derivatives were found. The oils compositions are shown in Table 2, and a representative chromatogram with the oil profile is presented in Figure 1.

Although plants were collected from the wild, and not subjected to control conditions, the edaphoclimatic conditions of the three populations were nearly the same. They were collected in different months (April and June) and years (2012 and 2013), but in the same season, autumn in south hemisphere, which is a dry period in the Cerrado area. Climate conditions, such as temperatures, length of the day and rain occurrence were the same for the areas of sampling, which were not far (approximately 50 km) from each other, as well as the soil type, red latosol. The populations collected were in the same vegetative stage, without flowers nor fruits.

The oils are rich in compounds with a terpinene-like structure, such as alpha and gamma terpinene, terpinolene, *p*-cymene, and the respective oxygenated compounds (terpineols, cymenols, and thymol), all closely related considering their biosynthetic pathway.

Table 2. Chemical composition of the essential oils of *P. laruotteanum*.

Peak	LRI[a] calc	LRI[b] lit	Identification	2410	2436	2605
1	925	924	α-thujene	0.1	0.1	*t*
2	932	932	α-pinene	13.4	11.6	9.2
3	947	953	Camphene	0.1	0.4	0.6
4	976	975	Sabinene	1.0	0.2	n.d.
5	990	991	Myrcene	1.0	0.6	0.6
6	1005	1005	α-phellandrene	1.9	1.6	0.2
7	1010	1011	δ-3-carene	1.2	1.1	1.4
8	1016	1014	α-terpinene	2.2	1.1	0.2
9	1023	1023	*p*-cymene	24.8	19.4	34.8
10	1026	1024	Limonene	3.0	10.2	7.9
11	1029	1026	1,8-cineole	19.2	6.9	12.5
12	1045	1044	(*E*)-β-ocimene	n.d.	1.4	n.d.
13	1056	1054	γ-terpinene	3.6	14.0	6.9
14	1070	1067	*cis* linalool oxide	n.d.	0.1	0.2
15	1087	1086	Terpinolene	3.6	5.1	0.9
16	1099	1097	Linalool	2.1	3.4	2.0
17	1111	1114	*endo*-fenchol	0.3	0.2	0.5
18	1124	1122	α-campholenal	n.d.	0.2	0.3
19	1135	1139	(*E*)-pinocarveol	0.8	0.1	0.2
20	1162	1162	Borneol	0.5	0.6	1.3
21	1174	1177	Terpinen-4-ol	6.3	5.8	2.7
22	1185	1183	*p*-cymen-8-ol	*t*	n.d.	0.4
23	1188	1189	α-terpineol	4.2	4.5	6.0
24	1193	1194	Myrtenol	n.d.	n.d.	0.5
25	1216	1218	Fenchyl acetate	*t*	0.1	0.4
26	1282	1283	Isobornyl acetate	*t*	1.7	1.4
27	1284	1289	Thymol	*t*	0.1	0.6
28	1290	1289	*p*-cymen-7-ol	*t*	0.3	0.6
29	1348	1346	α-terpinyl acetate	n.d.	1.7	n.d.
30	1371	1374	α-copaene	0.3	0.3	0.2
31	1413	1419	(*E*)-caryophyllene	0.6	*t*	n.d.
32	1447	1455	α-humulene	0.7	0.2	n.d.
33	1508	1514	γ-cadinene	0.2	0.3	*t*
34	1518	1523	δ-cadinene	0.6	0.6	0.2
35	1575	1578	Caryophyllene oxide	0.8	0.8	n.d.
36	1583	1590	Globulol	0.4	0.8	1.9
37	1584	1592	Viridiflorol	0.5	0.2	0.3
38	1586	1595	Cubeban-11-ol	0.1	0.1	0.7
39	1594	1600	Rosifoliol	*t*	0.3	0.6
40	1601	1608	Humulene epoxide II	0.9	n.d.	n.d.
41	1621	1629	1-*epi*-cubenol	0.8	0.8	0.5
42	1634	1640	*epi*-α-cadinol	0.5	1.5	0.6
43	1642	1646	α-muurolol	0.7	0.4	0.6
44	1645	1651	β-eudesmol	1.0	*t*	*t*
45	1647	1654	α-eudesmol	1.4	*t*	*t*
			Total:	98.8	98.8	97.9
			Total monoterpene hydrocarbons:	55.9	66.8	62.7
			Total oxygenated monoterpenes:	33.4	25.7	29.6
			Total sequiterpenes hydrocarbons:	2.4	1.4	0.4
			Total oxygenated sesquiterpenes:	7.1	4.9	5.2

[a]On a HP-5MS column, according to Ref. (9).
[b]According to Ref. (10); *t*: trace (<0.1%); n.d.: not detected.

It is interesting to observe that for the sample 2436, in which a lower content of *p*-cymene was recorded, higher concentrations of specifically other terpinene-like isomers were found (limonene, γ-terpinene and terpinolene). This seems to be related either to a more restrict biosynthetic profile, which points out to a lower genomic variability among the three populations, or to the effect of non-enzymatic and abiotic conditions. Albeit the last possibility has been demonstrated to occur with other plant families (11), the former is more likely to have occurred with the samples of *P. laruotteanum* analysed, since edaphoclimatic conditions were quite similar within the collection sites. Another difference that may impart the observed variation on the oil composition is that sample 2436 was harvested in a more disturbed area, while 2410 and 2605 were collected from well-preserved areas, which can might explain some different content in some specific compounds.

The oil composition of *P. laruotteanum* was quite different from the oils of other Brazilian *Psidium*. *P. myrsinites* DC., for instance, collected in the same area and season, yielded an oil rich in caryophyllene oxide, β-caryophyllene and other sesquiterpenoids, but not in monoterpenes (12). *P. myrsinoides* O. Berg, also from the Cerrado and a closely related species to *P. myrsinites*, yielded an oil rich in β-caryophyllene and its oxide too (13).

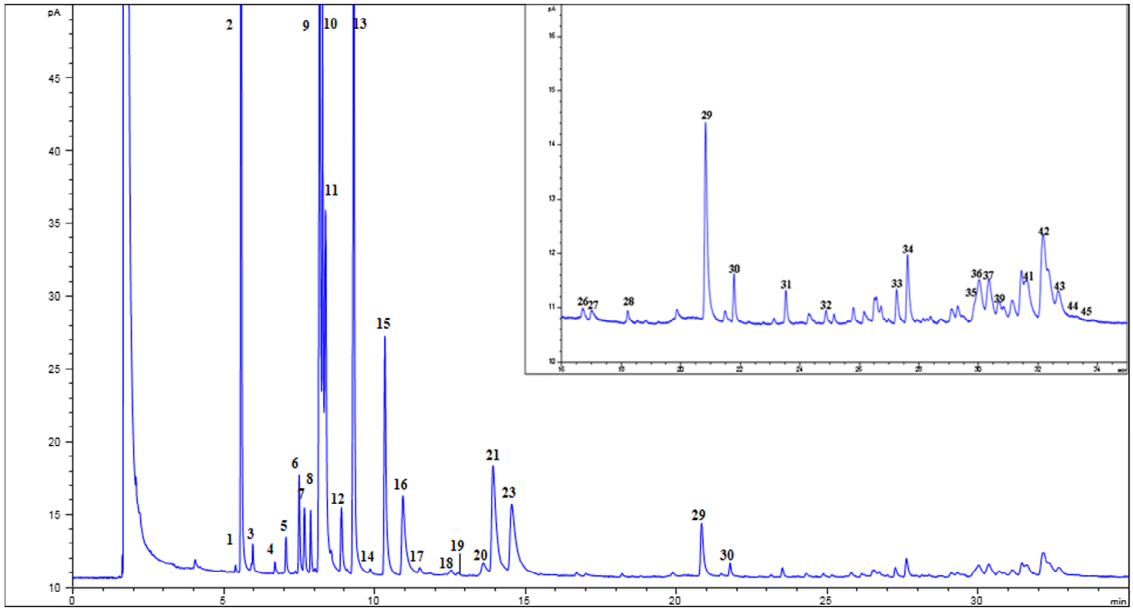

Figure 1. Representative chromatogram for the essential oil of *P. laruotteanum*.

Considering *Psidium* from different biomes, leaf oils from both *P. pohlianum* Berg and *P. guyanensis* Pers., from a semi-arid region (named *Caatinga*), were rich in 1,8-cineole (14). This compound was also found in high amounts in the oil of *P. cattleianum* Sabine, a species occurring in the Atlantic rainforest, although the major compound identified in this species was α-thujene (15). A study on *Psidium* from the Amazon (16) revealed oils rich in β-caryophyllene and α-selinene (*P. striatulum* Mart. ex DC.), β-bisabolol and limonene (*P. guineense* Sw.), α-pinene and 1,8-cineole (*P. acutangulum* DC., *P. guajava* L.).

P. guajava, guava tree is, by far, the most known and studied species of the genus. The essential oil from its leaves is rich in β-caryophyllene, although the compositions can vary greatly, which is not uncommon to a plant largely bred and cultivated (17).

Psidium is a genus largely distributed in Brazil, with sixty-six species, forty-four of them endemic and thirty-one found in the Cerrado biome. The species from the Cerrado are usually rich in sesquiterpenes, as shown by the data listed above. From our survey of aromatic plants from the Cerrado vegetation, it is quite unusual to find plants with predominance of monoterpenes. Still, albeit a great variability in oil profile has been described throughout the plants from different biomes, none of the *Psidium* oils investigated so far presented a similar composition to that of *P. laruotteanum*.

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