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ORIGINAL ARTICLE

Bioprospecting of strawberry guava leaf essential oil in Caxias do Sul region, South Brazil

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Abstract - The strawberry guava (*Psidium cattleianum* Sabine) is a fruit species native from South-Southeast Brazil whose leaf essential oil has potential for commercial uses due to its bioactive properties. However, little is known about the influence of the geographical distribution in small scale on *P. cattleianum* essential oil chemical composition and yield. The present work aimed to evaluate the yield and chemical composition of the leaf essential oil of *P. cattleianum* populations in the region of Caxias do Sul, South Brazil. Samples of twelve populations were collected and the essential oil was extracted by hydrodistillation. The chemical composition of the essential oils was determined by GC/MS and GC-FID. The results showed a high variability of essential oil yield, which ranged between 0.08 and 0.75% v/w. Regarding the chemical profiles, nine populations presented the 1,8-cineole chemotype, the β -caryophyllene chemotype was observed in two of them, and in one population there were four major compounds. Both hierarchical cluster and principal component analyses showed differences in essential oil composition in populations geographically close, indicating the existence of an important genetic variability in populations of the same geographical area.

Keywords: Native species. Chemotype. Terpenes. *Psidium cattleianum* Sabine. Genetic variability.

Bioprospecção do óleo essencial das folhas de araçá na região de Caxias do Sul, Sul do Brasil

Resumo - O araçá (*P. cattleianum*) é uma espécie originária do Sul-Sudeste do Brasil cujo óleo essencial apresenta potencial para aplicação comercial devido às propriedades bioativas. No entanto, pouco se sabe sobre a influência da distribuição geográfica em pequena escala em relação à composição química do óleo essencial desta espécie. O presente trabalho teve como objetivo avaliar o rendimento e a composição química do óleo essencial das folhas de *P. cattleianum* de diferentes populações da região do município de Caxias do Sul, Sul do Brasil. Amostras de doze populações foram coletadas e o óleo essencial foi extraído por hidrodestilação, a composição química dos óleos foi determinada por GC. Os resultados mostraram que nove populações apresentaram o quimiotipo 1,8-cineol, em duas o quimiotipo β -cariofileno foi observado e em uma população ocorreram quatro compostos majoritários. Tanto as análises de agrupamento hierárquico quanto de componentes principais mostraram diferenças na composição do óleo essencial em populações geograficamente próximas, indicando a existência de variabilidade genética importante nas populações de mesma área geográfica.

Palavras-chave: Espécies nativas. Quimiotipo. Terpenos. *Psidium cattleianum* Sabine. Variabilidade genética.

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Introduction

The strawberry guava or 'araçá', *Psidium cattleianum* Sabine (ITIS, 2022; WFO, 2022), also written *Psidium cattleyanum* (USDA, 2022), is a perennial tree native from South and Southeast Brazil and Uruguay, belonging to Myrtaceae family. Its fruits are appreciated locally, being consumed in natura or as an ingredient in jams, candies, and ice creams, among others. Besides the gastronomic potential, studies also report the antimicrobial and pharmacological properties of the strawberry guava fruits, leaves, bark, and roots (FETTER *et al.*, 2010; CHRYSTAL *et al.*, 2020; TAFAREL *et al.*, 2021).

P. cattleianum has essential oil in both the fruits and the leaves, whose composition and yield are variable and depend on the plant population, edaphoclimatic conditions, and stressing factors, both biotic and abiotic (PRINS;VIEIRA; FREITAS, 2010; SILVA *et al.*, 2021). Moreover, several studies addressed its antioxidant, antibacterial, and antifungal properties, among others (MARQUES *et al.*, 2008; CASTRO *et al.*, 2015; CHRYSTAL *et al.*, 2020; SAVOLDI *et al.*, 2020; SILVA *et al.*, 2021).

Due to the wide native geographical distribution of *P. cattleianum* and its easy adaptability to tropical and subtropical climates (CABI, 2019), there is a great variation among the chemotypes observed in the studied populations, as observed by Silva *et al.* (2021), who reported the existence of ten distinct chemical profiles in *P. cattleianum* populations, both in natural habitat and in introduced regions.

However, for a proper commercial exploitation of the essential oil of this species, it is important to carry out bioprospecting studies, both at a regional and greater levels, aiming to verify similarity patterns or differences in chemical composition and yield, which may or may not be linked to geographical isolation, environmental conditions, or due to stochastic genetic fluctuations (PRINS *et al.*, 2010; PANSEIRA *et al.*, 2021). This work aimed to evaluate the yield and chemical composition of the leaf essential oil of different *P. cattleianum* populations located in the region of Caxias do Sul, South Brazil.

Materials and Methods

Plant material sampling and essential oil obtainment

Plant material from *P. cattleianum* populations was collected from twelve sites, all in the municipality of Caxias do Sul, South Brazil, in April 2017. Vouchers of the collected populations were deposited in the Herbarium of the University of Caxias do Sul. The geographical coordinates and altitude of the collection sites are presented in Table 1.

Only green and healthy leaves, without damage or wilting signs were collected. The samples of five random plants of each site were sampled, totaling about 1 kg of fresh material for each population. The collected material was dried in a kiln with forced air circulation for 48 h at 30±5 °C, being kept away from moisture and sunlight.

The essential oil was extracted by hydrodistillation, using a 5 L glass round-bottom flask and a glass Clevenger type apparatus with a capacity of 3.00 mL and scale resolution of 0.05 mL. Heating was provided using an electrical heating mantle with maximum power of 690 W; the extraction was carried out using half-power (345 W). The extractions were carried out for 3 h, using 250 g of sample and 2.5 L of tap water in each





extraction (1:10 proportion). The extractions were carried out in triplicates. After extraction, the essential oil was separated from the hydrolate using a micropipette.

Table 1. Geographical coordinates and average altitude of the collection sites of *P. cattleianum* populations studied in this work.

Population	Geographical coordinates	Average altitude ¹ (m)
1-7	29°09' S; 51°08' W	800-825
8	29°11' S; 51°01' W	700
9	29°09' S; 51°13' W	735
10-12	29°08' S; 50°59' W	800-805

¹ – Average altitude above sea level.

Essential oil yield was determined following the procedures described by Pauletti *et al.* (2020). The obtained essential oil was stored in amber glass flasks and sent to chromatographic analysis.

Chromatographic analysis

The samples were analyzed by GC/MS (qualitative analysis) and GC-FID (quantitative analysis), in triplicate. The GC/MS analysis was carried out using a Hewlett-Packard 6890 gas chromatograph, coupled to a MSD5973 selective mass detector, equipped with the HP Chemstation software and the Wiley 275 spectra library. It was used an HP-5MS fused silica column (30 m x 250 µm i.d. and 0.5 µm film thickness). Temperature program was 60 °C (8 min) to 180 °C at 3 °C·min⁻¹ and up to 230 °C at 20 °C·min⁻¹. Injector temperature of 220°C, interface at 250 °C, split ratio 1:100, helium as carrier gas at 56 kPa and flow rate of 1.0 mL·min⁻¹, ionization energy of 70 eV.

The GC-FID analysis was carried out in a HP6980 gas chromatograph equipped with the HP Chemstation software, using an HP-5MS fused silica column (30 m x 250 µm i.d. and 0.5 µm film thickness). The temperature program was the same for GC/MS analysis, injector temperature of 250 °C, split ratio 1:50, flame ionization detector at 250 °C, using hydrogen as carrier gas at 34 kPa. Injected sample volume of 1.0 µL, diluted in hexane (1:10). Quantification was carried out by adding 1-octanol as internal standard, injecting 25 µL of a 30.22 g·L⁻¹ 1-octanol solution in hexane (755 µg of 1-octanol injected in each analysis).

Essential oil constituents were identified by comparison of the mass spectra with the ones in the Wiley spectra library (GC/MS) and by comparing the linear retention indexes (LRI) with literature data (ADAMS, 2017). The LRI values were calculated with the Van den Dool and Kratz equation, using a standard alkane solution (C8-C30) as reference. The determination of the mass percentages of each component was carried out using calibration curves for each chemical class (relative response factors) and the chromatogram peak area of each component, according to the procedures proposed by Rebelo *et al.* (2020).





Statistical analysis

The plant material of the five plants of each population were mixed and homogenized before drying, yielding a global sample for each population. After drying, each sample was divided in three subsamples, which were the triplicates used in essential oil extraction.

A hierarchical clustering analysis (HCA) and principal component analysis (PCA) were carried out using the data of essential oil chemical composition. The HCA was generated using the Ward's method and Euclidian distance. The PCA was generated using the covariance matrix of the data of the contents of all identified components, following the procedures proposed by Pansera *et al.* (2021).

Results and Discussion

The essential oil yield of the samples presented a wide disparity between populations, ranging between 0.08% (Population 1) and 0.75% v/w (Population 3), not being observed a relationship between the geographical microregion and the yield (Table 1). Chalannavar *et al.* (2012) reported an essential oil yield of 1.24% v/w for *P. cattleianum* leaves from South Africa, whereas Chrystal *et al.* (2020) reported 0.30 wt.% for plants in the region of Limeira, Southeast Brazil, and Savoldi *et al.* (2020) observed a yield of 0.83% v/w for *P. cattleianum* plants from Mangueirinha, Central-South Paraná state, South Brazil.

As addressed by Prins *et al.* (2010) and Pansera *et al.* (2021), essential oil yield is the parameter most influenced by environmental conditions, especially when they cause a stressing state in the plant. In situations where the environment is unfavorable there is an increase in the production of secondary metabolites as a defensive measure, such as in situations of drought or excessive rainfall, intense sunlight, attack of pests and herbivores and/or human intervention (PRINS *et al.*, 2010; PAULETTI *et al.*, 2020).

The chemical composition of the leaf essential oil of the studied *P. cattleianum* populations, as well as the essential oil yield, are compiled in Table 2.

According to Table 2, the oxygenated monoterpene 1,8-cineole was the major compound of the essential oil of most of the studied populations, except in populations 10, 11, and 12. β -caryophyllene was the major compound of the essential oils of the populations 10 and 11, and the oil of the population 12 presented an intermediate profile, having several major compounds with similar contents (1,8-cineole, β -caryophyllene, caryophyllene oxide, globulol, and α -cadinol).

According to the literature, *P. cattleianum* essential oil has great variation between populations and geographical location, the presence of sesquiterpenes whose molecules are based in caryophyllene skeleton is quite common (SILVA *et al.*, 2021), although there are reported populations whose essential oil was mainly composed by monoterpenes (MARQUES *et al.*, 2008), or a mixture of mono and sesquiterpenes, with the occurrence of composite chemotypes with two or more major compounds (SCUR *et al.*, 2016; CHRYSTAL *et al.*, 2020).

A Hierarchical Cluster Analysis was carried out to observe the degree of similarity and separation patterns of the different *P. cattleianum* populations based on the chemical composition of the leaf essential oils. The generated dendrogram is presented in Figure 1.





Table 2. Chemical composition (mean±SD; wt.%) of the leaf essential oil of different *Psidium cattleianum* populations (1-12), collected in Caxias do Sul region, South Brazil.

Compound	Calc. LRI	Lit. LRI	Populations											
			1	2	3	4	5	6	7	8	9	10	11	12
thujane	928	932	0.79±0.06	0.49±0.05	0.71±0.20	0.38±0.04	0.48±0.11	0.30±0.03	0.30±0.06	0.33±0.08	0.74±0.17	-	-	0.11±0.04
α-pinene	933	932	8.20±0.71	3.42±0.81	5.85±1.10	2.80±0.68	3.83±0.90	4.75±0.67	2.72±0.41	3.10±0.65	9.93±2.05	1.96±0.33	1.96±0.58	5.47±0.96
β-pinene	970	974	0.79±0.09	0.37±0.07	0.52±0.09	0.28±0.07	0.40±0.10	0.38±0.03	0.31±0.04	0.29±0.04	0.59±0.10	0.13±0.02	0.18±0.04	0.92±0.40
myrcene	988	988	2.90±1.06	1.40±0.59	2.24±0.79	1.31±0.38	1.37±0.69	0.75±0.12	1.23±0.37	1.11±0.22	1.96±0.35	3.69±0.75	4.15±0.56	2.24±0.84
α-phellandrene	1003	1002	0.17±0.04	0.29±0.08	0.37±0.08	0.29±0.10	0.22±0.08	0.35±0.04	0.28±0.05	0.32±0.09	0.47±0.07	-	-	0.17±0.06
δ-3-carene	1010	1008	0.20±0.06	-	-	-	0.11±0.02	0.19±0.04	0.10±0.02	-	-	-	-	0.07±0.01
α-terpinene	1014	1014	0.26±0.05	0.61±0.21	0.74±0.41	0.62±0.25	0.46±0.06	0.75±0.09	0.62±0.18	0.65±0.11	-	-	-	0.13±0.03
p-cymene	1021	1020	9.61±1.35	-	-	-	-	2.29±0.62	-	-	1.05±0.30	1.20±0.28	0.20±0.02	0.23±0.07
limonene	1026	1024	-	-	-	-	-	-	-	-	-	1.46±0.15	1.41±0.36	-
1,8-cineol	1027	1026	32.19±3.12	33.42±4.47	50.39±3.68	33.36±2.28	32.78±3.27	20.19±1.15	29.80±1.84	37.87±2.94	41.01±3.29	-	-	7.76±1.20
(Z)-β-ocimene	1033	1032	1.33±0.65	1.07±0.29	1.04±0.35	1.44±0.55	0.84±0.20	0.81±0.18	1.73±0.27	0.98±0.20	2.20±0.44	0.56±0.07	1.04±0.26	0.07±0.02
(E)-β-ocimene	1044	1044	0.32±0.08	0.32±0.12	0.32±0.09	0.43±0.12	0.24±0.03	0.28±0.06	0.51±0.10	0.54±0.09	0.61±0.18	0.15±0.03	0.43±0.10	-
γ-terpinene	1056	1054	2.40±0.94	2.88±0.69	3.82±0.66	2.87±0.94	2.29±0.40	4.56±0.72	3.40±0.70	3.61±0.77	5.27±0.69	0.14±0.03	0.85±0.26	0.29±0.05
terpinolene	1090	1086	0.38±0.12	0.33±0.04	0.44±0.21	0.34±0.02	0.31±0.04	0.45±0.03	0.42±0.09	0.42±0.05	0.46±0.09	1.30±0.32	3.33±0.69	0.11±0.04
linalol	1096	1095	5.24±0.84	6.25±1.02	9.29±1.75	7.45±1.33	7.08±1.25	10.95±1.89	8.52±2.15	10.83±1.45	4.22±1.07	4.94±0.49	3.36±0.35	2.64±0.33
terpinen-4-ol	1174	1174	1.78±0.44	2.28±0.35	2.35±0.48	2.29±1.04	2.16±0.53	1.66±0.33	2.63±0.51	3.20±0.87	0.83±0.20	1.26±0.55	0.89±0.31	0.77±0.18
α-terpineol	1188	1186	8.73±2.10	10.20±2.21	9.07±2.11	9.42±2.14	8.51±1.02	2.98±0.48	9.41±2.18	11.86±2.31	3.20±0.54	0.46±0.11	0.36±0.10	2.44±0.31
bornyl acetate	1280	1284	-	-	-	-	-	-	-	-	-	0.12±0.04	0.09±0.03	0.12±0.04
trans-pinocarvyl acetate	1299	1298	-	-	-	-	-	-	-	-	-	-	0.06±0.03	0.38±0.08
δ-terpinyl acetate	1315	1316	-	-	-	-	-	-	-	-	-	0.82±0.19	0.66±0.14	0.89±0.17
α-cubebene	1350	1345	0.24±0.38	0.16±0.03	-	-	0.24±0.03	0.06±0.01	0.28±0.04	0.43±0.07	0.24±0.10	0.16±0.05	0.17±0.02	-
neryl acetate	1358	1359	-	-	-	-	-	-	-	-	-	0.15±0.05	0.12±0.03	0.12±0.02
ylangene	1370	1373	-	-	-	-	-	-	-	-	-	0.56±0.20	0.41±0.13	0.42±0.09
β-copaene	1376	1374	2.03±0.66	3.65±0.55	0.92±0.22	3.99±0.68	4.12±0.97	5.06±0.97	3.92±0.39	0.79±0.10	-	1.20±0.39	1.74±0.62	3.17±0.87
β-maaliene	1381	1380	0.23±0.04	0.28±0.04	-	0.28±0.02	0.07±0.01	0.13±0.02	0.09±0.02	0.78±0.25	0.22±0.06	2.03±0.50	0.46±0.08	0.15±0.06
β-cubebene	1390	1387	-	-	-	-	-	-	-	-	-	0.43±0.10	0.36±0.06	0.49±0.08
α-gurjunene	1409	1409	-	-	-	-	-	-	-	-	-	-	-	0.12±0.02
β-caryophyllene	1420	1417	2.01±0.60	1.52±0.19	1.30±0.18	1.97±0.85	1.81±0.66	3.69±0.29	1.97±0.45	5.08±0.69	7.20±1.06	27.67±2.54	26.58±2.21	7.84±1.11
γ-gurjunene	1427	1431	-	-	-	-	-	-	-	-	-	0.59±0.15	0.74±0.20	0.99±0.28
γ-elemene	1438	1434	-	0.12±0.06	-	-	0.10±0.02	-	0.13±0.03	-	0.26±0.05	0.36±0.08	0.25±0.06	0.34±0.09
aromadendene	1440	1439	-	-	-	-	-	-	-	-	-	0.16±0.03	0.15±0.03	0.56±0.06
α-humulene	1450	1452	0.22±0.04	0.17±0.02	-	0.21±0.06	0.22±0.03	0.40±0.08	0.23±0.08	0.41±0.11	0.59±0.11	4.69±1.01	4.44±1.10	1.74±0.60
alloaromadendrene	1458	1458	-	-	-	-	-	-	-	-	-	0.43±0.10	0.31±0.07	0.85±0.20
β-muurolene	1480	1478	0.34±0.02	0.56±0.07	-	0.48±0.05	0.43±0.05	0.48±0.07	0.48±0.10	0.99±0.47	0.62±0.10	2.42±0.35	2.27±0.40	2.46±0.69
γ-selinene	1490	1489	0.67±0.18	1.11±0.27	0.45±0.15	1.01±0.61	0.86±0.22	1.14±0.32	0.97±0.33	1.67±0.84	1.29±0.48	0.32±0.10	1.88±0.29	0.32±0.06
(E)-methyl isoeugenol	1492	1491	0.32±0.09	0.67±0.11	-	0.52±0.12	0.66±0.15	0.69±0.14	0.72±0.08	0.48±0.10	-	1.54±0.35	1.27±0.47	0.93±0.09
valencene	1493	1496	0.26±0.06	0.24±0.03	-	0.31±0.04	0.35±0.08	0.47±0.06	0.34±0.06	0.88±0.14	0.98±0.16	1.71±0.29	1.79±0.60	0.36±0.10
α-selinene	1499	1498	0.19±0.10	0.21±0.06	-	0.18±0.07	0.24±0.06	0.32±0.05	0.26±0.10	0.68±0.11	0.80±0.18	1.37±0.30	1.21±0.023	3.38±0.55
(E)-β-guaiene	1504	1502	-	-	-	-	0.11±0.02	0.16±0.03	0.12±0.05	-	0.33±0.05	1.19±0.26	1.15±0.19	0.99±0.09
β-bisabolene	1507	1505	-	-	-	-	-	-	-	-	-	0.33±0.06	0.34±0.06	0.46±0.07
γ-cadinene	1509	1513	0.71±0.22	1.28±0.36	0.38±0.05	1.43±0.33	1.19±0.33	1.68±0.39	1.29±0.40	0.23±0.02	0.50±0.13	1.35±0.31	1.88±0.35	5.30±1.09
δ-cadinene	1522	1522	-	0.11±0.05	-	-	0.14±0.02	0.25±0.07	0.15±0.02	-	0.46±0.06	1.99±0.47	2.05±0.48	1.82±0.54
epiglobulol	1531	1532	-	-	-	-	-	-	-	-	-	0.23±0.05	0.12±0.03	0.46±0.09
nerolidol	1566	1561	3.56±0.67	2.54±0.33	1.38±0.40	2.46±0.70	4.16±0.88	4.18±0.77	3.29±0.38	0.98±0.34	1.11±0.25	1.00±0.23	1.20±0.29	1.24±0.37
caryophyllene oxide	1584	1582	1.82±0.94	3.11±0.50	0.90±0.27	2.83±0.45	2.28±0.55	4.30±0.83	2.46±0.50	1.49±0.67	1.67±0.14	6.41±1.12	5.35±0.66	7.43±1.53
globulol	1595	1590	-	-	-	-	-	-	-	-	-	-	-	7.43±1.88
jumperol	1600	1599	3.61±0.56	6.68±0.94	1.89±0.84	4.61±0.99	6.68±1.05	5.15±0.52	6.65±0.97	1.02±0.23	1.27±0.31	4.95±0.90	-	2.40±0.60
guaiol	1601	1600	0.28±0.02	0.64±0.06	-	0.48±0.05	0.71±0.21	1.08±0.30	0.68±0.11	-	0.28±0.04	1.29±0.44	1.78±0.45	0.65±0.11
lodal	1603	1602	1.05±0.25	-	0.51±0.09	-	-	-	-	0.43±0.15	1.84±0.56	-	-	1.55±0.37
γ-eudesmol	1633	1630	0.32±0.11	0.73±0.12	-	0.77±0.25	0.82±0.19	0.96±0.22	0.76±0.07	-	0.22±0.03	0.48±0.03	0.72±0.11	0.38±0.04
δ-cadinol	1648	1644	1.09±0.14	1.95±0.44	0.55±0.04	0.73±0.06	1.72±0.64	2.45±0.47	1.77±0.39	0.45±0.06	1.05±0.35	2.46±0.67	3.16±0.40	3.17±0.71
α-cadinol	1658	1652	-	-	-	-	-	-	-	-	-	-	6.34±1.47	7.02±2.25
α-bisabolol	1690	1685	-	-	-	-	-	-	-	-	-	0.76±0.22	1.03±0.22	2.63±0.48
juniper camphor	1702	1700	0.70±0.10	2.25±0.65	0.70±0.18	2.81±0.44	2.52±0.54	5.94±0.65	2.03±0.59	0.83±0.16	1.10±0.12	2.01±0.88	0.95±0.20	0.10±0.03
Monoterpenes			27.35	11.18	16.05	10.76	10.55	15.86	11.62	11.35	23.28	10.59	13.92	9.44
Oxygenated monoterpenes			48.26	52.82	71.10	53.04	51.19	36.47	51.08	64.24	49.26	9.29	6.81	16.05
Sesquiterpenes			6.90	9.41	3.05	9.86	9.88	13.84	10.23	11.94	13.49	48.96	48.18	31.76
Oxygenated sesquiterpenes			12.43	17.90	5.93	14.69	18.89	24.06	17.64	5.20	8.54	19.59	20.65	34.46
Total identified			94.94	91.31	96.13	88.35	90.51	90.23	90.57	92.73	94.57	88.43	89.56	91.71
Not identified			5.06	8.69	3.87	11.65	9.49	9.77	9.43	7.27	5.43	11.57	10.44	8.29
Yield (% v/w)			0.08±0.04	0.60±0.07	0.75±0.09	0.60±0.03	0.60±0.05	0.10±0.03	0.40±0.02	0.30±0.07	0.60±0.08	0.10±0.01	0.20±0.03	0.40±0.03

Calc. LRI: calculated linear retention index; Lit. LRI: linear retention index from Adams (2017).

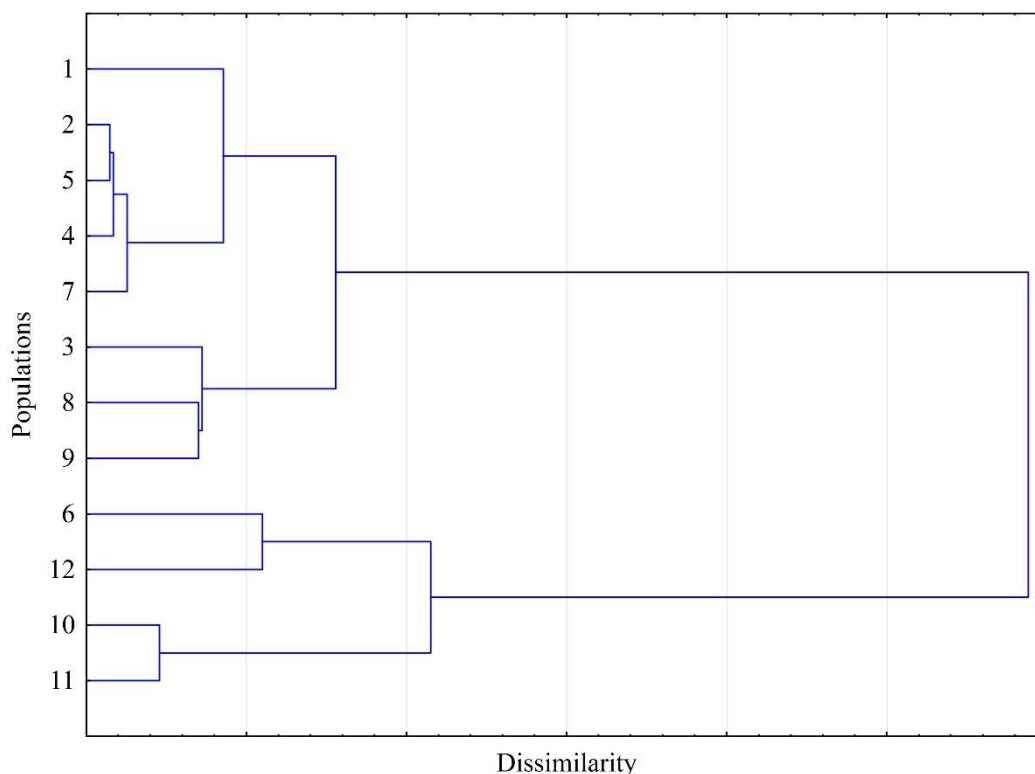


Figure 1. Dendrogram clustering the twelve studied *Psidium cattleianum* populations based on leaf essential oil Chemical composition (HCA carried out using Ward's method and Euclidian distance).

According to the dendrogram, it can be seen the separation of the populations in two major clusters, whose main difference was the content of 1,8-cineole. The first cluster, containing the populations 6, 10, 11, and 12 were characterized by the lower 1,8-cineole content. The subgroup of populations 6 and 12 had 1,8-cineole present in the oil and lower contents of β -caryophyllene. The essential oil of populations 10 and 11 had no 1,8-cineole present, had smaller amounts of α -terpineol, and β -caryophyllene was the major compound.

The cluster encompassing the populations 1 to 5 and 7 to 9 was characterized by 1,8-cineole as the major compound. The subgroup composed by populations 3, 8, and 9 had higher amounts of 1,8-cineole and similar juniperol contents. The subgroup formed by the populations 1, 2, 4, 5, and 7 had lower amounts of 1,8-cineole; the population 1 separated due to the presence of p-cymene, which was not present in the essential oils of the other populations in the same subgroup.

These variations found in populations geographically close may also be observed on a larger scale, as observed by Silva *et al.* (2021), who reported ten distinct chemical profiles for the leaf essential oil of *P. cattleianum* from several parts of the world. Considering only South and Southeast Brazil, five chemical profiles were reported, all containing a caryophyllene derivative as the major compound. Rocha *et al.* (2021) also noted differences in the composition of the essential oil of two morphotypes of *P. cattleianum* from South Brazil, whose major compound was 1,8-cineole. Chrystal *et al.* (2020), on the other hand, described a chemotype presenting viridiflorol, β -caryophyllene, 1,8-cineole, and β -selinene as the most abundant



compounds in the leaf essential oil of *P. cattleianum* plants from Paraná state, South Brazil.

Essential oil composition data also underwent Principal Component Analysis to verify the distribution of the populations relative to the chemical composition and the effects of the individual components on the variability of the essential oils extracted. The PCA results are presented in Figure 2.

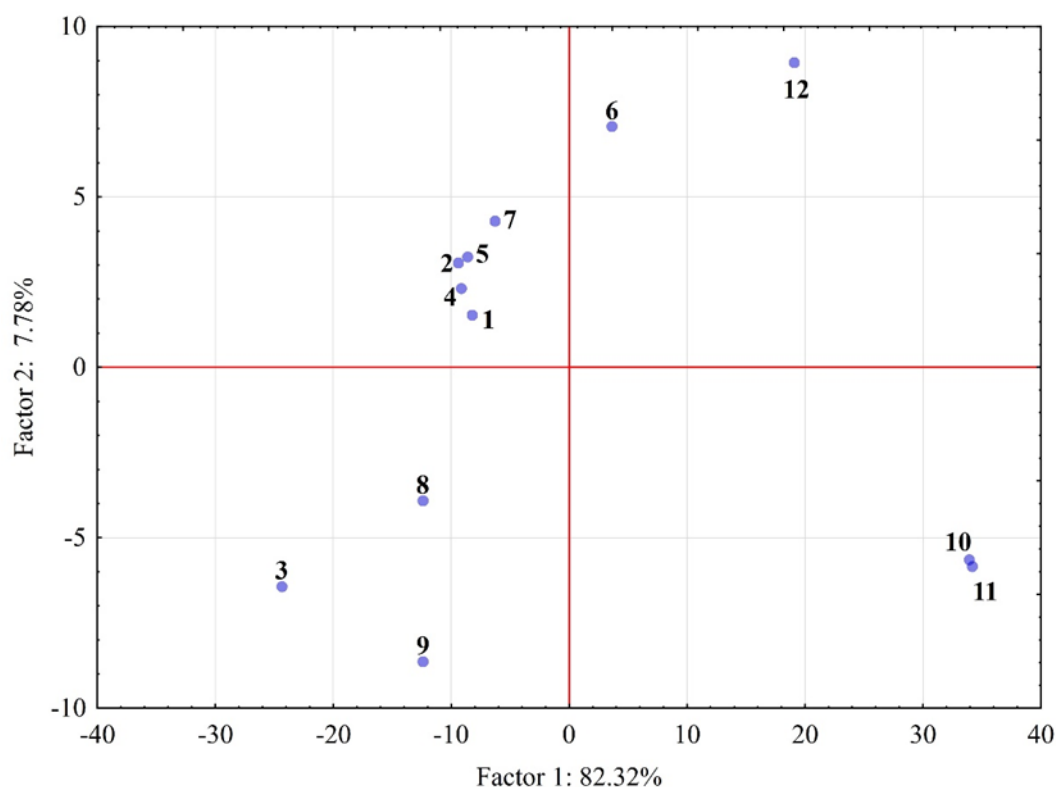


Figure 2. Principal Component Analysis (PCA) of the studied *Psidium cattleianum* populations based on the covariance matrix of the chemical composition of the leaf essential oil.

According to Figure 2, the chemical profiles divided themselves relative to the quadrants and clusters. The essential oils of the populations 1, 2, 4, 5, and 7 were clustered in the upper left quadrant, being characterized by similar and intermediate contents of 1,8-cineole (29.8-33.4 wt.%), and β -caryophyllene contents smaller than 2.0 wt.%.

The populations 6 and 12 were grouped in the upper right quadrant, having in common smaller amounts of 1,8-cineole (20.2 and 7.8 wt.%, respectively). The higher amount of 1,8-cineole in the essential oil of population 6 shifted it to the left in the diagram. It is also noteworthy observing that the essential of these two populations had β -caryophyllene contents higher than the other populations, excepting populations 10 and 11.

Populations 10 and 11 were grouped in lower right quadrant, being characterized by the absence of 1,8-cineole in the essential oil. These populations had β -caryophyllene as the major compound, being their essential oil composed mainly of sesquiterpenes; only population 12 also presented sesquiterpenes as the major chemical class that constituted the essential oil.

**Table 3.** Contribution of the identified compounds to PCA factors.

Compound	Contribution (%)	
	Factor 1	Factor 2
thujane	0.0147	0.0053
α -pinene	0.3220	0.3432
β -pinene	0.0009	0.0193
mircene	0.1468	0.8253
α -phellandrene	0.0031	0.0044
δ -3-carene	0.0000	0.0029
α -terpinene	0.0104	0.0186
p-cymene	0.0065	0.1828
limonene	0.0612	0.1949
1,8-cineole	71.3916	16.3506
(Z)- β -ocimene	0.0217	0.1327
(E)- β -ocimene	0.0017	0.0208
γ -terpinene	0.4379	0.1398
terpinolene	0.1007	0.4809
linalool	0.6765	0.2566
terpinen-4-ol	0.0713	0.0167
α -terpineol	3.2190	1.1661
bornyl acetate	0.0006	0.0000
<i>trans</i> -pinocarvyl acetate	0.0006	0.0067
δ -terpinyl acetate	0.0291	0.0002
α -cubebene	0.0008	0.0041
neryl acetate	0.0108	0.0002
ylangene	0.0000	0.0023
β -copaene	0.0001	0.0023
β -maaliene	0.0000	0.0010
β -cubebene	0.0001	0.0041
α -gurjunene	0.0000	0.0008
β -caryophyllene	19.6154	55.8853
γ -gurjunene	0.0073	0.0012
γ -elemene	0.0004	0.0111
aromadendene	0.0029	0.0075
α -humulene	0.6747	1.0186
alloaromadendrene	0.1100	0.0081
γ -muurolene	0.1836	0.0111
β -selinene	0.0000	0.0545
(E)-methyl isoeugenol	0.1022	0.0126
valencene	0.0475	0.3174
α -selinene	0.0064	0.0930
(E)- β -guaiene	0.0320	0.0211
β -bisabolene	0.0559	0.0001
γ -cadinene	0.1731	1.8489
δ -cadinene	0.0546	0.0425
epiglobulol	0.0290	0.0032
nerolidol	0.0027	1.9590
caryophyllene oxide	0.0029	1.1616
globulol	0.0389	3.1726
juniperol	0.9823	5.7804
guaiol	0.1291	0.0031
ledol	0.0264	0.0207
γ -eudesmol	0.0571	0.1000
δ -cadinol	0.1437	0.3314
α -cadinol	0.0793	0.4767
α -bisabolol	0.1830	0.1255
juniper camphor	0.0025	1.1535





In the lower right quadrant, the populations 3, 8, and 9 were grouped. The essential oils of these populations were characterized by the higher contents of 1,8-cineole (37.9-50.4 wt.%) and similar juniperol content (1.0-1.9 wt.%). However, the differences between the contents caused a sparser clustering, different from what was observed in the upper left and lower right quadrants.

Silva *et al.* (2021), evaluating the essential oil of *P. cattleianum* populations from several parts of Brazil, as well populations from United States, Cuba, and Egypt, reported more than ten distinct chemical profiles. However, the same authors commented that caryophyllene and its derivatives are present in variable contents, in addition of the presence of acyclic monoterpenes, as myrcene and pinenes. Other authors also cited the presence of caryophyllene derivatives as major compounds, although there are populations whose essential oil may present more than one major compound, characterizing a composite chemotype (CHALANNAVAR *et al.*, 2013; SCUR *et al.*, 2016; CHRYSTAL *et al.*, 2020; ROCHA *et al.*, 2021). Both monoterpenes and sesquiterpenes were observed as major compounds in the chemical profiles of *P. cattleianum* leaf essential oil from Brazil (CHRYSTAL *et al.*, 2020; ROCHA *et al.*, 2021; SILVA *et al.*, 2021). The individual contributions of each terpene to the PCA factors are compiled in Table 3.

According to Table 3, both PCA factors were mainly influenced by 1,8-cineole and β -caryophyllene contents, compounds that determined the chemotypes observed in the essential oil of the studied *P. cattleianum* populations. However, it is important to observe that Factor 2 also had a larger contribution from minor compounds, as the sesquiterpenes globulol and juniperol, indicating that this class of compounds may have a wider variability in relation to the content in the essential oil of different populations of this species. Silva *et al.* (2021) commented on the great variability in the chemical composition of the essential oil of *P. cattleianum* populations throughout the world, and although the β -caryophyllene chemotype and their derivatives are predominant, 1,8-cineol may also appear as a secondary (CHRYSTAL *et al.*, 2020; SILVA *et al.*, 2021), or major compound (ROCHA *et al.*, 2021).

There was high variability in the essential oil yield of the different populations, ranging from 0.08% to 0.75% v/w, and an average yield of 0.39% v/w. Relative to the observed chemotypes, most of the studied populations had 1,8-cineole as the major compound, whereas two populations presented β -caryophyllene as the major compound and absence of 1,8-cineole; One population had a mixed chemotype, with similar contents of 1,8-cineole, β -caryophyllene, and α -cadinol. This suggests that there are important genetic variations between the different *P. cattleianum* populations, even in a restricted geographical area.

Conflict of interest

The authors declare that the research was conducted in the absence of any potential conflicts of interest.

Ethical statements

The authors confirm that the ethical guidelines adopted by the journal were followed by this work, and all authors agree with the submission, content and transfer of the publication rights of the article to the journal.





They also declare that the work has not been previously published nor is it being considered for publication in another journal.

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