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Research Article

## Chemical composition of essential oils from six *Psiadia* species endemic to Madagascar Island

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### Abstract

The present study was carried out to compare the chemical composition of the leaf essential oils (EOs) from six *Psiadia* species endemic to Madagascar, namely *P. altissima*, *P. stenophylla*, *P. hispida*, *P. leucophylla*, *P. lucida* and *P. salviifolia*. Three of these species (*P. altissima*, *P. lucida* and *P. salviifolia*) are traditionally used for treating diarrhea, stomach ache and skin diseases. They are also known for their antiseptic, expectorant, hemostatic, carminative and anti-inflammatory properties. The EOs of the six species were obtained by hydrodistillation and analysed by capillary GC-FID and GC-MS. Their oil yields ranged from 0.04% to 0.80%. 149 components were identified, accounting for 84.0 to 97.9% of the total composition. The EOs from the four species *P. altissima*, *P. stenophylla*, *P. hispida* and *P. salviifolia* showed similar chemical composition which was dominated by monoterpene (63.1-78.4%) and sesquiterpene (5.7-25.7%) hydrocarbons. The major compounds identified in these oils were  $\beta$ -pinene (17.2-46.5%), limonene (10.4-28.5%) and (*Z*)- $\beta$ -ocimene (5.4-7.3%). The two other species exhibited qualitative and quantitative differences in the chemical composition of their EOs. The essential oil (EO) from *P. lucida* was found to be rich in sesquiterpene hydrocarbons (46.6%) and oxygenated sesquiterpenes (32.0%).  $\alpha$ -cadinol (11.1%),  $\alpha$ -muurolene (10.6%) and  $\delta$ -cadinene (7.5%) were the main components. The *P. leucophylla* EO was characterized by a prominent content of sesquiterpene hydrocarbons (66.4%). The main compounds included  $\beta$ -caryophyllene (10.6%),  $\delta$ -cadinene (8.9%) and (*E*)-muurola-4(14),5-diene (7.2%). This was the first report on the chemical composition of the EOs from *P. stenophylla*, *P. hispida* and *P. leucophylla*.

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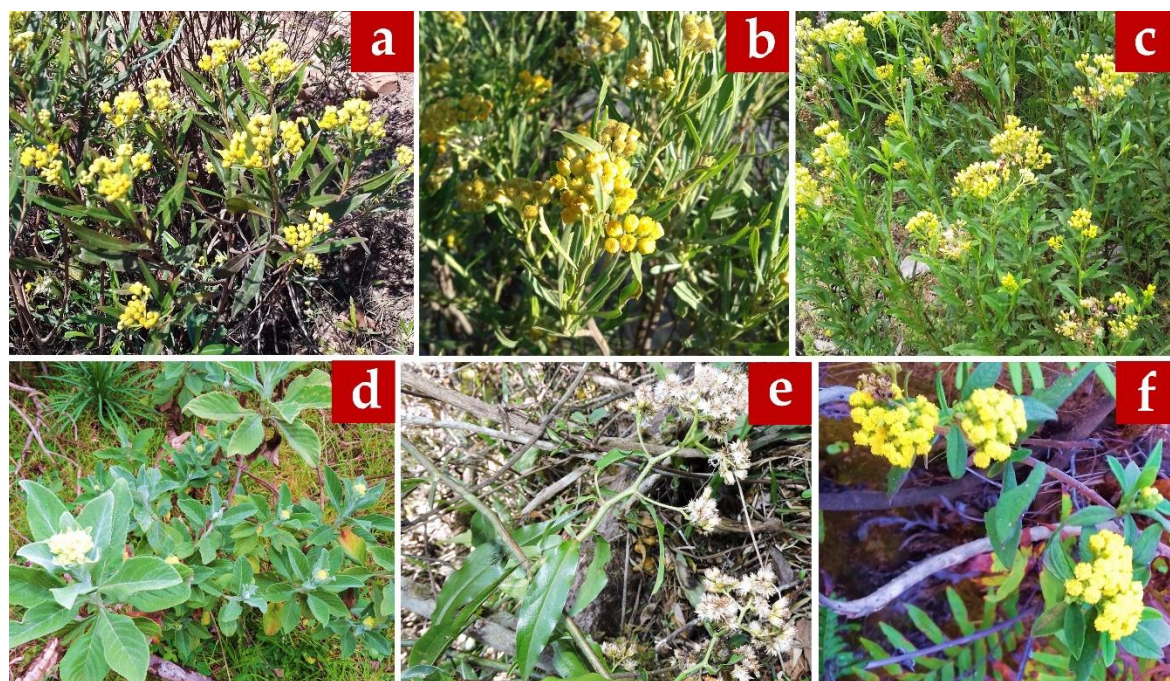
### Keywords

*Psiadia*, essential oils, chemical composition, Madagascar, GC-MS, GC-FID

## 1. Introduction

The genus *Psiadia* belonging to the *Asteraceae* (*Compositae*) family includes about 60 species. One species is present in Indonesia and Sri Lanka, several species in Arabia and Africa (tropical Africa and South Africa) [1], 26 species in the Mascarene Islands (Reunion Islands, Mauritius and Rodrigues) [2] and

28 species in Madagascar [3]. Some *Psiadia* species have been traditionally used for a long time to treat a variety of ailments such as abdominal pains [4], cold, fever [5, 6], bronchitis and asthma [7]. They are also used in casts for broken bones by the Bedouins [8] or to treat minor wounds and burns [9]. *P. punctulata*



**Figure 1.** Pictures of the six *Psiadia* species investigated: **a-** *P. altissima* (PAL), **b-** *P. stenophylla* (PST), **c-** *P. hispida* (PHI), **d-** *P. leucophylla* (PLE), **e-** *P. lucida* (PLU) and **f-** *P. salviifolia* (PSA).

roots have been used by the Maasai to fight malaria [10]. *P. altissima*, *P. salviifolia* and *P. lucida* are three well-known species in the Malagasy pharmacopeia. Their leaves are notably used to treat tooth [3,11,12], and stomach aches [13] and skin diseases like scabies, eczema and wounds [11,12,14–16]. They are also recognised for their hemostatic, anti-diarrheal, carminative, anti-hypertensive and disinfectant properties [17–21].

As reported in a recent paper [22], several *Psiadia* species produce EOs [9,17,21,23–26] and contain various specialized metabolites including flavonoids [8, 27–29], terpenoids [23,30–32], phenylpropanoids [28,33–35] and coumarins [27, 36]. These metabolites displayed a variety of biological effects notably antimicrobial and antifungal [5, 6, 17, 24, 25, 37, 38], antiviral [39–43], antiplasmodial [30, 44–46], anti-inflammatory [36, 39, 47], cytotoxic [48, 49] and wound healing activity [38].

Considering the medicinal potential of the genus *Psiadia*, extensive investigation on six species endemic to Madagascar Island was undertaken. The chemical composition of the EOs from *P. altissima*, *P. stenophylla*, *P. hispida*, *P. leucophylla*, *P. lucida* and *P. salviifolia* was studied. EO components from three of them, namely *P. stenophylla*, *P. hispida* and *P. leucophylla* were characterized for the first time.

## 2. Materials and methods

### 2.1 Plant materials

Leaves of the six *Psiadia* species (Fig. 1) were collected in Madagascar, during their flowering periods (October-January). The species were botanically identified by the botanists of the National Herbarium of the Tsimbazaza Botanical and Zoological Garden in Antananarivo (Madagascar). Each collected specimen was deposited both in the national Herbarium of the Tsimbazaza Botanical and Zoological Garden and in the Herbarium of the University of Reunion. The date of harvest, collection site, herbarium voucher number, and geographic coordinates are listed in Table 1.

### 2.2 Essential oil extraction

After 24h of collection, the EOs were separately extracted from the leaves of each species by hydrodistillation for three hours using a Clevenger-type apparatus. The recovered oils were dried over anhydrous sodium sulphate  $\text{Na}_2\text{SO}_4$  and kept at 4 °C until analysis. The extraction yields were calculated based on the weight of fresh plant material and reported in Fig. 2.

### 2.3 Gas chromatography analysis

The total chemical constituents were identified using the GC Agilent Technologies 6890 Network, equipped with mass spectrometer (MS) Agilent Technologies 5973 Network, with a SPB-5 MS fused-silica capillary



**Table 1.** Date of harvest, collection site, herbarium voucher number, and geographic coordinates for the *Psiadia* specimens.

Species	Date of harvest	Collection site	M/car voucher number	Reunion voucher number	Coordinates Latitude/Longitude	Altitude (m)
<i>Psiadia altissima</i>	02 Nov. 2018	Moramanga	RIR 3196	REU024089	18°58'16.7"S/48°19'16.7"E	919
<i>Psiadia stenophylla</i>	31 Oct. 2018	Behenjy	RIR 3189	REU024090	19°15'22.7"S/47°28'53.0"E	1 395
<i>Psiadia hispida</i>	14 Jan. 2019	Angavokely	RZK 8265	REU024086	18°55'38.1"S/47°44'13.2"E	1 758
<i>Psiadia leucophylla</i>	02 Nov. 2018	Angavobe	RIR 3193	REU024087	18°55'34.9"S/47°45'07.8"E	1 743
<i>Psiadia lucida</i>	14 Jan. 2019	Andasibe	RIR 3195	REU024082	18°55'18.8"S/48°25'30.9"E	967
<i>Psiadia salviifolia</i>	30 Oct. 2018	Antsirabe	RIR 3185	REU024085	19°52'32.7"S/47°05'18.7"E	1 648

m: meters; M/car: Madagascar

column (60 m × 0.32 mm i.d., 0.25 µm film thickness). Helium was used as the carrier gas at a flow rate of 0.7 mL/min. The pure EO was injected with a volume of 0.01 µL in a split ratio of 1:50 to enhance sensitivity and minimize peak broadening. The injection was performed manually. Oven temperature was programmed from 40 °C to 250 °C at 4 °C/min, and held at 250 °C for 50 min. Injector and source temperatures were set at 250 °C and 280 °C, respectively. The GC-MS instruments were operated at 70 eV in the Electronic Ionisation (EI) mode and mass spectra (MS) were scanned in the range 20-400 Daltons.

Quantitative data regarding the volatile constituents were performed by using GC Varian CP-3800, coupled to a flame ionization detector (FID). The analysis is operating at the same conditions as GC-MS, except for the helium flow rate which was set at 1.0 mL/min. The injected volume of pure essential oil was 0.01 µL in splitless mode to ensure the complete transfer of the sample. The FID detector temperature was set at 270 °C.

**2.4 Identification and quantification of compounds**

The retention index (RI) for all volatile components of the EOs was calculated using a homologous series of C<sub>8</sub>-C<sub>28</sub> n-alkanes as standards. The identification of volatile components was carried out by comparing their retention index and their mass spectral fragmentation pattern with the literature [50,51] and data stored in MS libraries (Wiley 07 and NIST 02).

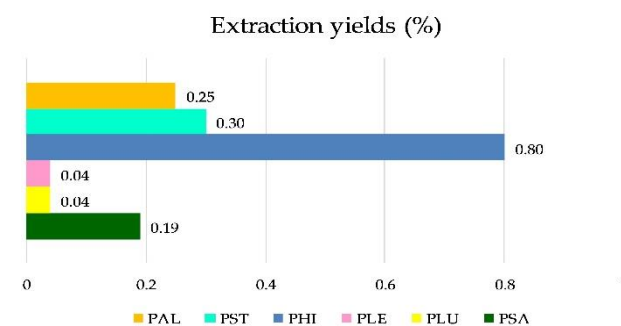
The relative amount of each identified compound, expressed as a percentage (%), was calculated by comparing the area of the corresponding peak in the chromatogram with the total area of detected peaks, without using FID correction factors. This approach allowed for the quantification of each compound in the EOs, based on their peak areas in the

chromatogram.

**3. Results and discussion**

**3.1 Essential oil yields**

The EOs from the leaves of the six *Psiadia* species were obtained as a pale yellow to dark yellow liquid. Oil yields, calculated from the weight of fresh plant material, ranged from 0.04% to 0.80% as shown in Fig. 2, which is typical in *Psiadia* species [17, 21, 23, 24, 52, 53].



**Figure 2.** Essential oil yields obtained from the leaves of *Psiadia* species: *P. altissima* (PAL), *P. stenophylla* (PST), *P. hispida* (PHI), *P. leucophylla* (PLE), *P. lucida* (PLU) and *P. salviifolia* (PSA).

In an earlier study, oil yield ranging from 0.13% to 0.70% was reported for *P. altissima* [17, 26] and 0.18% for *P. salviifolia* [21]. Our results (0.25% for *P. altissima* and 0.19% for *P. salviifolia*) were consistent with these values which are typical to the two *Psiadia* species. EOs from *P. lucida* and *P. leucophylla* were obtained in 0.04% yield. This was a poor value compared to the literature value of 0.91% for *P. lucida* [25] which means that the oil yield of *P. lucida* may be growing site dependent. *P. stenophylla* and *P. hispida* exhibited the highest values of oil yield, 0.30% and 0.80% respectively. Interestingly, the oil yield of most *Psiadia* species from different origins did not exceed 1% [17, 23, 24, 52, 53].

### 3.2 Chemical composition

The chemical composition of six *Psiadia* species was investigated using both GC-MS and GC-FID techniques. Identification was performed by correlating their retention index with their mass spectra. Table 2 lists the components identified in their EOs, their RI, and their concentration in the different samples.

A total of 149 compounds were identified for the six *Psiadia* species EOs, representing from 84.0 to 97.9% of the total composition. These compounds are divided into 10 chemical families, including monoterpene hydrocarbons, oxygenated sesquiterpenes and aromatic compounds. Fig. 3 illustrates how the constituents in the EO of each species are distributed among the different chemical families.

The chemical composition analysis of the EOs from the six *Psiadia* species has revealed a diverse array of chemical families. This diversity highlighted the complex nature of these EOs and underscored the potential for unique aromatic and therapeutic properties and applications. In the following discussion, the individual characteristics of each *Psiadia* species, their chemical profiles and their potential target application areas will be explored.

#### 3.2.1 *Psiadia altissima*

In the chemical compositions of *P. altissima* EO, monoterpene hydrocarbons were identified as the main constituents, comprising 73.4% of the composition, followed by sesquiterpene hydrocarbons (16.2%). Although there were 26 sesquiterpene hydrocarbons in the sample, they were only present at a concentration of 4.2%. In this EO, the major compounds were  $\beta$ -pinene (44.9%), limonene (10.4%), (*Z*)- $\beta$ -ocimene (7.3%) and  $\alpha$ -pinene (4.5%). Previous studies [17,26] have already investigated the chemical composition of *P. altissima* EO. They have found that the leaves of this species were rich in both monoterpene and sesquiterpene hydrocarbons, with  $\beta$ -pinene (39.7-49.7%), limonene (3.8-9.3%), (*E,Z*)- $\beta$ -ocimene (5.9-6.9%), and  $\alpha$ -pinene (3.5-3.9%) as major compounds. Our study on the chemical composition of *P. altissima* EO aligned with these established findings. Additionally, sesquiterpene hydrocarbons were detected, including  $\beta$ -caryophyllene (1.7%),  $\alpha$ -humulene (1.3%), (*Z*)- $\beta$ -guaiene (3.5%) and  $\delta$ -cadinene (1.7%), which was consistent with the earlier studies [17,26]. In contrast to the findings of these

previous investigations, ledene (3.9%), cyperene (3.4%), viridiflorene (3.0%) and aromadendrene (1.2%) were not observed in the present study.

#### 3.2.2 *Psiadia stenophylla* and *Psiadia hispida*

*P. stenophylla* and *P. hispida*, both investigated for the first time, displayed a chemical composition relatively close to that of *P. altissima*. Their EOs are characterized by a significant concentration of monoterpene hydrocarbons (67.9% and 78.4% respectively) as well as sesquiterpene hydrocarbons (20.7% and 5.7% respectively). Similar to *P. altissima* EO, the main compounds in these two EOs were  $\beta$ -pinene (34.9-46.9%), limonene (15.8-16.0%), (*Z*)- $\beta$ -ocimene (5.4-5.7%) and  $\alpha$ -pinene (4.2-4.7%). Furthermore, their EOs included identical sesquiterpene hydrocarbons, comprising  $\beta$ -caryophyllene,  $\alpha$ -humulene, and  $\delta$ -cadinene. However, in *P. stenophylla*,  $\gamma$ -curcumene (5.8%),  $\alpha$ -humulene (3.7%), and (*E*)- $\beta$ -guaiene (2.3%) were found in higher quantities compared to *P. altissima*, where they were either absent or occurred in lower amounts.

#### 3.2.3 *Psiadia leucophylla*

In this initial investigation of *P. leucophylla*, 84.0% of the EO chemical compositions were identified, with several minor compounds that remained unidentified. *P. leucophylla* exhibited significant dissimilarities in its chemical composition compared to the other species investigated. A diverse array of chemical families was observed in the EO, which was predominantly composed of sesquiterpene hydrocarbons (66.4%). Additionally, it contained 6.4% of oxygenated diterpenes and 6.2% of oxygenated sesquiterpenes. In contrast to the other species, *P. leucophylla* had notably very low levels of hydrocarbon monoterpenes (0.6%). The EO also contained other compound families but in minimal quantities, such as alcohols (0.4%), aldehydes (0.2%), sulphurated sesquiterpenes (0.1%), and trace amounts of aromatic compounds. The major compounds in this EO included  $\beta$ -caryophyllene (10.6%),  $\delta$ -cadinene (8.9%), (*E*)-muurola-4(14),5-diene (7.2%),  $\alpha$ -humulene (6.9%), and manool (6.4%). Additionally, the EO also showed a significant amount of  $\alpha$ -copaene (5.0%),  $\beta$ -copaene (4.6%),  $\gamma$ -muurolene (4.6%), and  $\alpha$ -muurolene (3.6%).

#### 3.2.4 *Psiadia lucida*

The EO of *P. lucida* has revealed high levels of sesquiterpene hydrocarbons (46.6%) and oxygenated sesquiterpenes (32.0%). Several compounds were

**Table 2.** Essential oils chemical composition of the six *Psiadia* species: *P. altissima* (PAL), *P. stenophylla* (PST), *P. hispida* (PHI), *P. leucophylla* (PLE), *P. lucida* (PLU) and *P. salvifolia* (PSA).

N°	Compound	RI (exp)	RI (lit)	Composition (%)					
				PAL	PST	PHI	PLE	PLU	PSA
<i>Alcohols</i>									
1	(3E)-hexenol	857	854	-	-	-	0.4	0.2	-
<i>Aldehydes</i>									
2	(2E)-hexenal	853	855	tr	-	-	-	-	-
3	n-octanal	1004	999	-	-	-	0.2	-	-
<i>Monoterpene hydrocarbons</i>									
4	α-thujene	931	930	0.1	-	0.6	-	-	0.1
5	α-pinene	940	939	4.5	4.7	4.2	-	-	2.4
6	camphene	956	954	2.9	4.8	-	-	-	-
7	sabinene*	980	975	0.4	-	46.9	-	0.5	1.4
8	β-pinene*	987	979	44.9	34.9	-	tr	-	17.2
9	myrcene*	993	991	-	0.1	-	-	2.1	-
10	α-phellandrene	1011	1003	tr	tr	-	-	-	2.1
11	p-mentha-1(7),8-diene	1017	1004	tr	-	-	-	-	0.5
12	α-terpinene*	1022	1017	tr	tr	1.2	-	-	3.2
13	p-cymene*	1030	1025	0.4	1.2	-	-	tr	-
14	limonene*	1035	1029	10.4	15.8	16	-	0.4	28.5
15	β-phellandrene*	1037	1030	-	-	-	-	3	-
16	(Z)-β-ocimene	1039	1037	7.3	5.4	5.7	0.6	0.5	2.7
17	(E)-β-ocimene	1050	1050	2.3	0.6	3	-	-	-
18	γ-terpinene	1064	1060	tr	-	-	-	-	2.1
19	terpinolene	1095	1089	0.3	0.5	0.5	-	-	2.9
20	p-1,3,8-menthatriene	1126	1103	-	-	0.3	-	tr	-
21	allo-ocimene	1132	1132	0.1	-	-	-	-	-
<i>Oxygenated monoterpenes</i>									
22	(Z)-sabinene hydrate	1075	1070	-	-	-	-	-	0.4
23	linalol	1103	1095	1.8	0.7	-	1.3	0.1	tr
24	(E)-sabinene hydrate	1107	1098	-	-	0.1	-	-	tr
25	α-fenchol	1123	1117	tr	0.1	-	-	-	-
26	(Z)-p-menth-2-en-1-ol	1130	1122	tr	-	tr	-	-	tr
27	(E)-p-menth-2-en-1-ol	1148	1141	-	-	tr	-	-	tr
28	borneol	1177	1169	0.1	0.2	-	-	-	-
29	terpinen-4-ol	1186	1177	0.7	0.4	2.7	-	0.1	1
30	α-terpineol	1199	1189	1.3	1.1	0.6	-	tr	0.2
31	fragranol	1219	1216	-	-	-	0.1	-	-
32	(E)-carveol	1227	1217	-	-	-	-	-	tr
33	nerol	1234	1230	0.1	-	-	tr	-	-
34	thymol methyl ether	1239	1235	-	-	-	-	tr	-
35	geraniol	1258	1253	0.2	-	-	0.1	0.2	-
36	bornyl acetate	1293	1289	tr	1	-	-	-	-
37	carquejol acetate	1306	1299	0.1	-	-	tr	-	-
38	(Z)-dimethoxy-citral	1326	1318	tr	-	-	-	-	-
39	(E)-carvyl acetate	1341	1342	-	-	-	-	-	0.1
40	nerol acetate	1365	1362	-	-	-	0.3	-	-
41	verbanol acetate	1372	1344	-	-	tr	-	-	-
42	(E)-myrtenyl acetate	1388	1387	-	-	0.1	-	-	-
43	(Z)-jasmone	1417	1393	-	-	-	-	-	0.1
44	neryl acetone	1457	1436	-	-	-	tr	-	-
<i>Sesquiterpene hydrocarbons</i>									
45	silphiperfol-5-ene	1338	1329	-	-	-	-	-	0.1
46	δ-elemene	1347	1338	0.7	-	0.2	0.1	0.1	0.6
47	7-epi-silphiperfol-5-ene*	1357	1348	-	-	-	-	-	0.6
48	α-cubebene*	1359	1351	tr	0.1	-	0.8	0.2	-
49	silphiperfol-4,7(14)-diene	1370	1361	-	-	-	-	-	0.1

Table 2. (continued)

N°	Compound	RI (exp)	RI (lit)	Composition (%)					
				PAL	PST	PHI	PLE	PLU	PSA
50	$\alpha$ -ylangene	1384	1375	0.1	0.1	-	-	tr	-
51	$\alpha$ -copaene	1388	1377	0.4	0.5	-	5	0.4	0.3
52	$\beta$ -maaliene	1397	1382	-	-	-	-	-	0.8
53	7- <i>epi</i> -sesquithujene	1397	1391	-	0.1	-	-	-	-
54	$\beta$ -bourbonene*	1398	1388	-	-	-	-	4.1	-
55	$\beta$ -cubebene*	1402	1388	-	-	-	-	-	-
56	$\beta$ -elemene*	1402	1391	0.6	1.0	0.7	-	-	1.2
57	cyperene	1418	1399	-	tr	-	-	-	-
58	$\alpha$ -gurjunene*	1424	1410	tr	0.1	-	0.2	0.1	0.1
59	$\beta$ -cedrene*	1427	1421	-	-	-	-	-	-
60	longifolene	1433	1408	tr	-	-	-	-	-
61	$\beta$ -ylangene*	1434	1421	-	-	-	tr	2.5	-
62	$\beta$ -caryophyllene*	1436	1419	1.7	2.3	1.0	10.6	-	2.9
63	$\beta$ -copaene	1444	1432	-	0.3	-	4.6	1.1	-
64	$\gamma$ -elemene	1444	1437	-	-	-	-	-	-
65	$\beta$ -gurjunene	1444	1434	0.7	-	-	-	tr	1
66	$\alpha$ -guaiene	1450	1440	-	-	0.1	-	-	-
67	aromadendrene	1460	1441	-	-	0.1	-	tr	-
68	(Z)-muurola-3,5-diene*	1462	1450	0.9	-	-	3.2	tr	0.6
69	(E)-muurola-3,5-diene*	1465	1454	-	0.3	-	-	-	-
70	$\alpha$ -humulene	1471	1455	1.3	3.7	1.7	6.9	2.9	1.4
71	(Z)-muurola-4(14),5-diene	1478	1467	0.2	0.1	tr	1.5	0.9	0.3
72	(E)-cadina-1(6),4-diene	14 87	1477	tr	-	tr	-	-	-
73	$\gamma$ -curcumene	1489	1483	0.7	5.8	-	-	-	2.2
74	$\gamma$ -muurolene	1491	1480	0.3	-	0.3	4.6	2.4	0.3
75	$\alpha$ -amorphene	1494	1485	-	-	-	-	-	-
76	germacrene D	1498	1485	0.9	1.0	-	-	-	-
77	$\beta$ -selinene	1504	1490	0.7	-	-	-	-	1.4
78	(E)-muurola-4(14),5-diene	1509	1494	0.2	0.6	0.1	7.2	0.9	2.3
79	$\alpha$ -muurolene	1513	1500	-	-	tr	3.6	10.6	-
80	(E)- $\beta$ -guaiene	1513	1503	-	2.3	-	-	6.9	-
81	(Z)- $\beta$ -guaiene	1514	1494	3.5	0.5	-	-	-	1
82	$\gamma$ -amorphene	1514	1496	-	-	-	-	-	1.4
83	$\beta$ -humachalene	1520	1505	-	0.6	-	-	-	-
84	$\alpha$ -bulnesene	1520	1510	-	-	0.3	-	-	-
85	$\delta$ -amorphene	1521	1512	0.2	-	-	2.4	1	0.9
86	$\gamma$ -cadinene	1528	1514	0.7	0.6	0.2	2.6	3.1	1.1
87	$\delta$ -cadinene	1537	1523	1.7	0.3	1	8.9	7.5	3.3
88	zonarene	1541	1530	tr	0.1	-	-	-	0.7
89	(E)-cadina-1(2),4-diene	1547	1535	0.1	tr	0.1	0.2	0.4	-
90	$\alpha$ -cadinene	1552	1539	0.2	tr	-	1	0.2	-
91	$\alpha$ -calacorene	1552	1546	-	0.4	-	2.6	0.4	1.2
92	germacrene B	1577	1561	0.2	-	-	-	0.8	0.1
93	$\beta$ -calacorene	1580	1566	-	-	-	0.1	-	-
94	cadalene	1693	1677	-	-	-	0.2	-	-
<i>Oxygenated sesquiterpenes</i>									
95	<i>epi</i> -cubebol	1509	1494	-	-	-	-	3.6	1
96	cubebol	1531	1515	tr	-	0.2	2.6	tr	0.5
97	10- <i>epi</i> -cubebol	1554	1535	-	-	-	-	-	2.8
98	elemol	1563	1550	0.4	0.6	-	-	3.5	0.3
99	(E)-nerolidol	1569	1563	0.3	0.2	0.1	tr	-	-
100	(E)-cadinene ether	1587	1559	-	-	-	-	-	0.1
101	ledol	1588	1569	-	0.2	-	-	1.1	-

Table 2. (continued)

N°	Compound	RI (exp)	RI (lit)	Composition (%)					
				PAL	PST	PHI	PLE	PLU	PSA
102	(Z)-muurol-5-en-4- $\alpha$ -ol*	1591	1561	-	-	-	-	-	0.2
103	germacrene D-4-ol*	1592	1576	-	-	tr	tr	tr	-
104	spathulenol	1597	1578	-	-	-	0.2	0.3	-
105	guaiol	1610	1601	tr	0.1	-	-	-	-
106	gleenol	1600	1587	-	-	tr	-	-	-
107	caryophyllene oxide	1603	1583	-	-	-	0.1	0.2	0.2
108	(Z)- $\beta$ -elemone	1618	1590	-	-	-	-	-	-
109	viridiflorol	1625	1593	-	0.2	-	0.4	1.6	0.3
110	humulene epoxyde II	1630	1608	-	-	-	-	0.5	-
111	$\beta$ -copaen-4- $\alpha$ -ol	1631	1591	-	-	-	-	-	0.1
112	1,10-di- <i>epi</i> -cubenol	1633	1619	tr	0.1	-	0.2	tr	-
113	10- <i>epi</i> - $\gamma$ -eudesmol	1642	1624	tr	0.1	-	-	tr	-
114	1- <i>epi</i> -cubenol	1647	1629	0.1	0.2	tr	tr	0.5	0.1
115	$\gamma$ -eudesmol	1651	1632	0.2	0.2	-	-	1.0	-
116	$\tau$ -cadinol*	1658	1640	0.2	0.3	-	-	-	-
117	$\tau$ -muurolol*	1661	1642	0.1	-	0.2	1.3	7.3	0.7
118	$\alpha$ -muurolol*	1664	1646	-	0.2	-	-	-	-
119	$\beta$ -eudesmol*	1672	1651	0.8	0.6	-	-	11.1	tr
120	$\alpha$ -cadinol*	1674	1654	0.2	-	0.4	1.2	-	0.4
121	$\alpha$ -eudesmol	1677	1654	0.1	0.2	-	-	-	-
122	<i>epi</i> - $\beta$ -bisabolol*	1681	1672	-	-	-	-	-	-
123	selin-11-en-4- $\alpha$ -ol*	1683	1660	0.1	0.3	-	0.1	-	0.1
124	(E)-14-hydroxy-9- <i>epi</i> -caryophyllene	1690	1670	-	-	-	-	-	0.1
125	<i>epi</i> - $\alpha$ -bisabolol	1695	1685	-	tr	-	-	-	-
126	$\alpha$ -bisabolol	1697	1686	-	tr	-	-	-	-
127	khusinol	1706	1680	-	-	tr	-	-	-
128	eudesma-4(15),7-dien-1- $\beta$ -ol	1706	1688	-	-	-	0.3	0.1	-
129	(E)- $\alpha$ -bergamotol	1711	1691	-	-	-	-	-	0.1
130	oplopanone*	1760	1740	-	-	-	-	-	-
131	cyclocolorenone*	1763	1761	-	-	-	-	0.4	-
132	14-oxy- $\alpha$ -muurolene	1787	1769	-	-	-	-	0.3	-
133	14-hydroxy- $\alpha$ -muurolene	1793	1780	-	-	-	tr	0.2	-
134	bicyclovetivenol	1815	1793	-	-	-	-	0.4	-
135	14-hydroxy- $\delta$ -cadinene	1820	1804	-	-	-	-	tr	-
136	(Z)-lanceol acetate	1964	1856	-	-	-	-	-	tr
<b>Sulfurized sesquiterpenes</b>									
137	mint sulfide	1764	1741	-	-	-	0.1	-	-
<b>Diterpene hydrocarbons</b>									
138	rimuene	1925	1896	-	-	-	-	-	tr
139	pimaradiene	1987	1950	tr	-	-	0.9	-	-
140	cembrene	2004	1939	-	-	-	-	-	0.1
141	sclarene	2071	1975	-	-	-	0.9	-	-
142	kaurene	2078	2043	-	-	-	-	-	0.1
<b>Oxygenated diterpenes</b>									
143	13- <i>epi</i> -oxyde de manol	2046	2017	-	-	-	-	-	0.4
144	manol	2082	2057	tr	-	0.4	6.4	-	tr
145	phytol	2116	1943	tr	tr	0.1	-	tr	-
146	nezukol	2230	2133	-	-	-	tr	-	-
<b>Aromatic compounds</b>									
147	perillene	1118	1101	-	0.3	-	-	tr	-
148	methyl eugenol	1408	1404	-	tr	-	-	-	-
149	benzyl benzoate	1783	1760	-	-	-	tr	0.1	-



Table 2. (continued)

N°	Compound	RI (exp)	RI (lit)	Composition (%)					
				PAL	PST	PHI	PLE	PLU	PSA
Total alcohols				-	-	-	0.4	0.2	-
Total aldehydes				tr	-	-	0.2	-	-
Total monoterpene hydrocarbons				73.4	67.9	78.4	0.6	6.4	63.1
Total oxygenated monoterpenes				4.2	3.4	3.5	1.8	0.3	1.8
Total sesquiterpene hydrocarbons				16.2	20.7	5.7	66.4	46.6	25.7
Total oxygenated sesquiterpenes				2.5	3.4	0.9	6.2	32	6.7
Total sulfurized sesquiterpenes				-	-	-	0.1	-	-
Total diterpene hydrocarbons				tr	-	-	1.8	-	0.1
Total oxygenated diterpenes				tr	tr	0.5	6.4	tr	0.4
Aromatic compounds				-	0.3	tr	tr	0.1	tr
<b>Total identified</b>				<b>96.3</b>	<b>95.8</b>	<b>89.0</b>	<b>84.0</b>	<b>85.6</b>	<b>97.9</b>

RI (exp): experimental retention index; RI (lit): retention index from the literature [50,51]; tr = trace, less than 0.1%; -: absent. \* Co-eluting compounds; [ ] : % of co-eluting compounds.

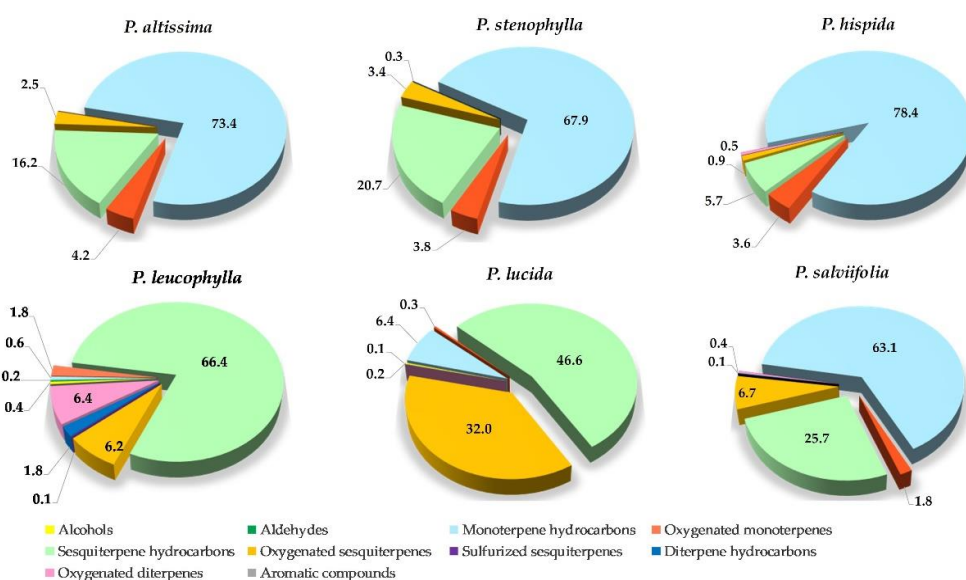


Figure 3. Distribution of EO constituents in *Psidium* species categorized by chemical families.

identified in relatively significant amounts, including  $\alpha$ -cadinol (11.1%),  $\alpha$ -muurolene (10.6%),  $\delta$ -cadinene (7.5%),  $\tau$ -muurolol (7.3%) and (Z)- $\beta$ -guaiene (6.9%). Notably, the results from this study showed a qualitative difference compared to the previous research conducted on *P. lucida* [25]. In this earlier study, terpinolene (38.0%),  $\alpha$ -humulene (21.2%), and limonene (10.2%) were identified as the main compounds [25], which differed from the current results. These findings suggest that the chemical profile of *P. lucida* EO may have varied depending on the harvest date and/or collection location.

### 3.2.5 *Psidium salviifolia*

*P. salviifolia* chemical compositions were characterized by a high proportion of monoterpene hydrocarbons (63.1%) and sesquiterpene hydrocarbons (25.7%). The

most abundant compounds were  $\beta$ -pinene (17.2%) and limonene (28.5%). This composition was relatively close to that of *P. altissima*, *P. stenophylla* and *P. hispida*. These results were consistent with previous research by Andrianarison [13], which reported monoterpene hydrocarbons (76.7%) as the predominant chemical family in the EO of *P. salviifolia*. The major compounds identified in that study included  $\beta$ -pinene (22.1%), limonene (19.9%),  $\beta$ -phellandrene (10.4%), and sabinene (9.7%). However, the present study did not corroborate the results of the initial investigation of this species by Dennis [21]. According to Dennis, EO was primarily composed of a mixture of linalool and  $\beta$ -bourbonene (10.7%), along with significant amounts of oxygenated sesquiterpenes (25.8%) and diterpenes (11.8%).

In our study, the major compounds identified were  $\beta$ -pinene (17.2%) and limonene (28.5%), which were also reported by Dennis but at relatively lower levels (4.6 % and 2.1%, respectively).

In previous studies, several major compounds that are present in *Psiadia* species EOs have been highlighted for their biological activities. Specifically, studies have highlighted the strong effects of compounds such as (+)- $\alpha$ -pinene and (-)- $\alpha$ -pinene [54–57], (-)- $\beta$ -pinene [58] and (+)- $\beta$ -pinene [59], (+)-limonene [60, 61] and (-)-limonene [61],  $\beta$ -caryophyllene [62] and  $\delta$ -cadinene [63, 64] against pathogenic yeasts and bacteria. Because of the hemostatic and disinfectant properties of these compounds, our findings therefore validated the traditional uses of *P. altissima*, *P. lucida*, and *P. salviifolia* species in the treatment of various wounds such as scabies, eczematous, ulcerated, and syphilitic. Our findings also validated their potential as a remedy against a variety of microbial infections, including diarrhea [18]. It is important to determine the enantiomeric composition of the studied essential oil as it significantly influenced its biological activity and potential therapeutic effects. Additionally, previous studies on *P. altissima*, *P. lucida* and *P. salviifolia* EOs have demonstrated their antimicrobial activities against Gram-positive strains, including *Staphylococcus aureus*, *Sarcina lutea*, *Enterococcus faecalis* and *Bacillus subtilis*, as well as Gram-negative strains such as *Escherichia coli*, *Salmonella enteridis*, *Salmonella typhi*, *Shigella boydii* and *Branhamella catarrhalis* [13, 17, 25, 38]. Furthermore, *P. altissima* and *P. salviifolia* EOs have exhibited antifungal properties against *Candida albicans* [13,38].

Moreover, the EO from *P. altissima* leaves has been specifically studied for its *in vivo* wound-healing activity [38]. In this study, significant wound-healing properties were revealed, with 98.37% healing after 14 days of treatment, which was close to the activity of a standard reference ointment. This aligned with the traditional use of this species for treating wounds [11,14]. This activity may be attributed to the presence of specific compounds detected in the EO, notably  $\alpha$ -pinene enantiomers and  $\beta$ -caryophyllene, both of which have been previously shown to have wound-healing properties [65, 66].

According to the literature, the main compounds highlighted in the studied EOs, notably  $\alpha$ -pinene

[67,68], limonene [69,70],  $\alpha$ -humulene [71], and  $\beta$ -caryophyllene [62, 72]) have also displayed significant anti-inflammatory and antioxidant potential. These results supported the traditional uses of *P. altissima*, *P. lucida* and *P. salviifolia* species, especially in remedying ailments such as bronchitis, cough and asthma, potentially due to their ability to reduce respiratory system inflammation. Furthermore, Rakotomalala reported that the EOs from the aerial parts of *P. altissima* had strong broncho-relaxant properties [38].

Most of the predominant compounds found in our EOs have demonstrated cytotoxic and antitumor properties, including  $\alpha$ -pinene [73],  $\beta$ -pinene [74], limonene [75], (*Z*)- $\beta$ -ocimene [76],  $\beta$ -caryophyllene [62],  $\alpha$ -cadinol [77], and manool [78]. These findings suggest that the six *Psiadia* EOs may have broader applications in the treatment of various ailments and potentially even in addressing issues related to abnormal tissue growth. This highlights the significance of further research and suggests the potential for harnessing the rich medicinal properties of *Psiadia* species to develop novel therapeutic agents for modern healthcare, particularly by further exploring their cytotoxic properties.

Beyond these categories, the six *Psiadia* EOs contained several major compounds with a diverse range of biological activity. Antileishmanial activity has been demonstrated for (*Z*)- $\beta$ -ocimene [76].  $\beta$ -pinene [79] and  $\alpha$ -humulene [80] both had a protective role against gastric injury. This may support the lengthy decoction of *P. altissima* leaves and stems that were prescribed in traditional pharmacopoeia to cure ulcerated wounds [18]. Antimalarial effects of  $\alpha$ -pinene and  $\beta$ -caryophyllene have been reported [81]. Furthermore,  $\alpha$ -pinene has shown therapeutic effect in ovalbumin (OVA)-sensitized allergic rhinitis [82]. These compounds could play roles in traditional applications, possibly in treating colds and fever.

#### 4. Conclusions

In the current investigation, the chemical composition of EOs extracted from fresh leaves of six *Psiadia* species endemic to Madagascar was evaluated. This was the first report on the volatile phytochemicals found in *P. stenophylla*, *P. hispida*, and *P. leucophylla*. This research revealed that the EOs from four species,

namely *P. altissima*, *P. stenophylla*, *P. hispida*, and *P. salviifolia*, had a similar chemical composition. Their EOs were dominated by monoterpenes and sesquiterpenes hydrocarbons. In contrast, the remaining two species, *P. lucida* and *P. leucophylla*, displayed distinct chemical profiles. The EO of *P. lucida* was rich in sesquiterpene hydrocarbons and oxygenated sesquiterpenes. *P. leucophylla* was characterized by sesquiterpene hydrocarbons and oxygenated diterpenes. These results not only contributed to the chemical knowledge of some *Psiadia* species but also lent support to their traditional uses and biological potential. Moreover, this finding offered a promising outlook for potential applications and therapeutic properties associated with the major compounds in these species, emphasizing the importance of further exploration into the multifaceted benefits that these EOs may offer.

### Authors' contributions

Conceptualization, L.E.R., A.G.B., I.G. and J.R.; Methodology, L.E.R., A.G.-B., I.G. and J.R.; Resources, L.E.R., A.R. (plant collections); Chemical investigation (extraction and analysis), L.E.R.; Writing-original draft preparation, L.E.R.; Writing-review and editing, L.E.R., A.G.-B., I.G., A.R. and J.R.; Supervision, A.G.B., I.G. and J.R.; Project administration and funding acquisition, A.G.B.

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### Availability of data and materials

All data will be made available on request according to the journal policy.

### Conflicts of interest

The authors declare no conflict of interest.

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