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Qualitative, Quantitative, and Enantioselective Chemical Profiling of Leaf Essential Oils from Ecuadorian Species *Piper peltatum* L. and *Piper umbellatum* L.

Karem Cazares [1,2], Yessenia E. Maldonado [3], Walter A. Palacios [4], Omar Malagón [1], Fani Tinitana [5] and Gianluca Gilardoni [1,*]

[1] Departamento de Química y Producción, Universidad Técnica Particular de Loja (UTPL), Calle París s/n y Praga, Loja 110107, Ecuador; kcazares@uea.edu.ec (K.C.); omalagon@utpl.edu.ec (O.M.)
[2] Departamento de Matemáticas y Ciencias Físicas, Universidad Estatal Amazónica, via Napo Km. 2½, paso Lateral s/n, Puyo 160150, Ecuador
[3] Programa de Doctorado en Química, Universidad Técnica Particular de Loja (UTPL), Calle Paris s/n y Praga, Loja 110107, Ecuador; yemaldonado2@utpl.edu.ec
[4] Investigador Asociado del Instituto Nacional de Biodiversidad (INABIO), Av. Río Coca E6-115 e Isla Fernandina, Quito 170501, Ecuador; walterpalacios326@yahoo.com
[5] Departamento de Ciencias Biológicas y Agropecuarias, Universidad Técnica Particular de Loja (UTPL), Calle Paris s/n y Praga, Loja 110107, Ecuador; ftinitana@utpl.edu.ec
* Correspondence: ggilardoni@utpl.edu.ec or gianluca.gilardoni@gmail.com

Abstract

This study describes the chemical composition of the essential oils (EOs) obtained from the leaves of *Piper peltatum* L. and *Piper umbellatum* L. collected in the Ecuadorian Amazon, together with the enantiomeric characterization of the volatile fraction of *P. umbellatum*. Qualitative (GC–MS) and quantitative (GC–FID) analyses were performed using two columns with stationary phases of different polarities: one nonpolar and the other polar. In total, 31 and 38 components were identified, respectively, in *P. peltatum* and *P. umbellatum* EOs. Both volatile fractions were dominated by sesquiterpenes, which accounted for 75.2–75.3% of the whole oil mass in *P. peltatum* and 80.1–78.2% in *P. umbellatum* on the nonpolar and polar phases, respectively. The major constituents of *P. peltatum* EO were (*E*)-β-caryophyllene (25.0–24.9%), γ-muurolene (22.1–22.4%), *n*-heneicosane (8.8–8.7%), bicyclogermacrene (7.7–7.5%), caryophyllene oxide (4.8–4.5%), (*E*)-nerolidol (4.0–3.8%), and α-copaene (2.9–3.1%). In *P. umbellatum*, the predominant compounds were γ-muurolene (30.5–31.2%), (*E*)-β-caryophyllene (13.0–12.4%), bicyclogermacrene (8.9–7.3%), δ-cadinene (5.2–5.4%), and limonene (4.0–3.8%). The enantioselective analysis of *P. umbellatum* EO allowed the characterization of five chiral metabolites, revealing a nearly racemic distribution for α-pinene and varying degrees of enantiomeric enrichment for camphene, β-pinene, limonene, and camphor. Taken together, these results expand our understanding of the chemical and stereochemical diversity of the EOs from these two *Piper* species from the Ecuadorian Amazon.

Keywords: Piperaceae; gas chromatography; mass spectrometry; enantiomer separation; Amazon forest

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1. Introduction

Plants have historically been one of the most important sources of organic compounds, mainly secondary metabolites, used in the development of drugs, fragrances, and other biologically and commercially relevant products [1–3]. Among the most important natural

products, essential oils (EOs) must be mentioned. According to the European Pharmacopoeia, EOs are "odorous products, usually of complex composition, obtained from a botanically defined plant raw material by steam distillation, dry distillation, or a suitable mechanical process without heating" [4].

With these premises, the study of natural products, in general, and EOs, in particular, is more promising in places where biodiversity is high, such as Ecuador. In fact, the United Nations Environment Programme's World Conservation Monitoring Centre recognized Ecuador as one of the 17 megadiverse countries worldwide, a status attributed to its extraordinary species richness and high levels of endemism. In terms of flora, Ecuador hosts more than 5000 endemic species, reflecting its exceptional botanical diversity [5]. However, despite this remarkable biodiversity, phytochemical investigations of many native species remain limited and fragmented [6,7].

In the Ecuadorian Amazon, local communities have traditionally relied on medicinal plants as part of their ethnobotanical knowledge, reflecting a close relationship with the natural environment and underscoring the potential of these species as sources of bioactive compounds of scientific interest [8]. For these reasons, *Piper peltatum* L. and *Piper umbellatum* L. were selected within the framework of a project, entitled "*Realizing the potential of plant bioresources as new economic opportunities for the Ecuadorian Amazon: developing climate resilient sustainable bioindustry*". This project is funded by British institutions, and it aims to identify six aromatic species from the Ecuadorian Amazon forest, with a potential for the sustainable production of EOs in favour of the indigenous communities.

The genus *Piper* (Piperaceae) is widely distributed throughout the tropical regions of the world. It is classified among the aromatic taxa due to the presence of oil cells distributed throughout various structures of its species [9]. Several species of the genus *Piper* have attracted considerable phytochemical interest due to the diversity of their secondary metabolites, the biological activities associated with these compounds, and the richness in EOs [10–12].

According to recent data, Piperaceae is a pantropical family, comprising five genera and 3000–3600 species. The largest genera are *Piper* and *Peperomia*, counting with about 2000 and 1600 species respectively [13]. Geographically, this family is widely distributed within the Americas, Africa, Asia, and Oceania, with Ecuador hosting most of the species (322), followed by Peru (288) and Bolivia (106) [14].

P. peltatum and *P. umbellatum* are neotropical species, distributed from Mexico to South America. *P. umbellatum* is also present in the Antilles and has been introduced to Africa and Southeast Asia [15,16].

From a taxonomic standpoint, *P. peltatum* and *P. umbellatum* have a complex nomenclatural history and have been described under different names over time [17–19].

In the field of ethnobotany, both species have a long history of traditional medicinal use. In the Ecuadorian Amazon, *P. peltatum* and *P. umbellatum* are known with vernacular names, such as "natsamak" and "Santa María" respectively [20]. In some Amazonian regions, *P. peltatum* has been traditionally used to treat inflammation, rheumatism, abdominal pain, fever, and eye conditions [21]. *P. umbellatum*, on the other hand, is widely used in the Americas, Africa, and Asia, for the treatment of gastrointestinal disorders, skin conditions, inflammatory processes, wounds, and rheumatism [16].

Previous studies have reported the chemical composition of essential oils (EOs) obtained from *P. peltatum* and *P. umbellatum* specimens collected in different geographical regions of South and Central America. These investigations have revealed considerable differences in the relative abundance of their major constituents, suggesting marked intraspecific chemical variability. The chemical profiles previously reported are therefore briefly introduced here and more extensively compared with those obtained in the present

study in the Section 3 [22–24]. However, the previously reported chemical profiles are quite different from the ones observed in the present study. Furthermore, no complete studies have been published about EOs from specimens collected in Ecuador. Finally, although some enantioselective analyses have already been reported for other *Piper* spp., no enantiomer separation was described for *P. peltatum* or *P. umbellatum* volatile fractions [25,26].

Therefore, to the best of the authors' knowledge, this study represents the first complete chemical analysis of a leaf EO distilled from Ecuadorian specimens of *P. peltatum* and *P. umbellatum*, as well as the first enantioselective analysis of a volatile fraction obtained from *P. umbellatum*.

2. Results

2.1. Chemical Composition of the EOs

The leaves of *P. peltatum* and *P. umbellatum* afforded two EOs upon steam distillation, characterized by yields of 0.26% and 0.50% on a dry-weight basis, respectively. Both EOs were characterized by a pleasant herbaceous aroma—somewhat pungent if concentrated—typical of many sesquiterpene-based volatile fractions. The chemical analyses were performed using two columns with stationary phases of different polarity: one nonpolar and the other polar. In the EO of *P. peltatum*, 36 components were detected, of which 31 were identified by comparison of their electron-impact mass spectra (EIMS) and linear retention indices (LRIs) with the literature data; five EO components remained unidentified. In *P. umbellatum*, 41 components were detected, of which 38 were identified and three remained unidentified. The quantified compounds accounted for 99.5–98.1% of the total EO mass for *P. peltatum* and 99.6–97.1% for *P. umbellatum* on the nonpolar and polar phases, respectively.

The volatile fractions of both species consisted mainly of sesquiterpenes, which accounted for 75.2–75.3% of the EO of *P. peltatum* and 80.1–78.2% of the EO of *P. umbellatum*. In *P. peltatum*, oxygenated sesquiterpenoids constituted the second most abundant group (14.1% and 12.0%), while other compounds accounted for 9.7% and 9.9%. In contrast, *P. umbellatum* also contained a significant proportion of monoterpenes (9.3% and 9.0%), followed by oxygenated sesquiterpenoids (7.0% and 6.5%).

The major constituents (≥3.0% in at least one column) of *P. peltatum* were (*E*)-β-caryophyllene (25.0–24.9%, **15**), γ-muurolene (22.1–22.4%, **23**), *n*-heneicosane (8.8–8.7%, **56**), bicyclogermacrene (7.7–7.5%, **26**), caryophyllene oxide (4.8–4.5%, **40**), (*E*)-nerolidol (4.0–3.8%, **37**), and α-copaene (2.9–3.1%, **13**). In *P. umbellatum*, the major components were γ-muurolene (30.5–31.2%, **23**), (*E*)-β-caryophyllene (13.0–12.4%, **15**), bicyclogermacrene (8.9–7.3%, **26**), δ-cadinene (5.2–5.4%, **32**), and limonene (4.0–3.8%, **7**).

The comparative chromatographic profiles of the two EOs, obtained using the nonpolar and polar stationary phases, are shown in Figures 1 and 2 respectively. The chemical structures of the major constituents (≥3.0% in at least one column and one oil) are shown in Figure 3. The complete results of the qualitative and quantitative analyses are presented in Table 1.

Table 1. Chemical composition of the EOs of *P. peltatum* and *P. umbellatum* using 5% [27] phenyl methyl polysiloxane and polyethylene glycol stationary phases. Major components (≥3.0% in at least one species and one column) are reported in bold. Standard deviations refer to the quantitative variations on the four analytical replicates.

No.	Compounds	5%-Phenyl Methyl Polysiloxane							Polyethylene Glycol						
		LRI		*P. peltatum*		*P. umbellatum*		Literature	LRI		*P. peltatum*		*P. umbellatum*		Literature
		Calc.	Ref.	%	σ	%	σ		Calc.	Ref.	%	σ	%	σ	
1	α-pinene	932	932	-	-	1.6	0.04	[27]	1012	1013	-	-	1.5	0.03	[28]
2	camphene	945	946	-	-	0.9	0.04	[27]	1049	1050	-	-	0.9	0.03	[29]
3	β-pinene	972	974	-	-	2.5	0.07	[27]	1098	1097	-	-	2.4	0.04	[30]
4	myrcene	991	988	-	-	0.2	0.03	[27]	1148	1148	-	-	0.3	0.03	[31]
5	*p*-mentha-1(7),8-diene	1006	1003	-	-	trace	-	[27]	1130	1137	-	-	trace	-	[32]
6	*p*-cymene	1016	1020	-	-	0.2	0.02	[27]	1253	1253	-	-	trace	-	[33]
7	**limonene**	1020	1024	-	-	4.0	0.09	[27]	1180	1180	-	-	3.8	0.10	[34]
8	camphor	1134	1142	-	-	1.5	0.03	[27]	1488	1490	-	-	1.5	0.04	[35]
9	δ-elemene	1329	1335	-	-	2.1	0.05	[27]	1454	1452	-	-	1.7	0.04	[36]
10	α-cubebene	1341	1348	1.4	0.08	1.0	0.06	[27]	1440	1449	1.5	0.09	0.9	0.01	[37]
11	unidentified (MW = 204)	1355	-	-	-	2.2	0.03	-	1550	-	-	-	2.1	0.05	-
12	α-ylangene	1363	1373	-	-	0.3	0.01	[27]	1462	1465	-	-	0.4	0.02	[36]
13	**α-copaene**	1368	1374	2.9	0.15	2.5	0.04	[27]	1470	1471	3.1	0.11	2.5	0.07	[34]
14	β-cubebene	1384	1387	2.7	0.15	1.8	0.02	[27]	1518	1522	2.8	0.06	1.5	0.04	[37]
15	**(*E*)-β-caryophyllene**	1408	1417	25.0	1.32	13.0	0.28	[27]	1572	1579	24.9	1.39	12.4	0.40	[28]
16	β-copaene	1419	1430	1.0	0.06	1.8	0.03	[27]	1566	1565	1.4	0.19	2.1	0.10	[38]
17	aromadendrene	1429	1439	0.6	0.04	0.2	0.01	[27]	1581	1580	0.6	0.15	0.5	0.08	[39]
18	unidentified (MW = 204)	1435	-	0.6	0.03	-	-	-	1461	-	0.3	0.08	-	-	-
19	α-humulene	1444	1452	2.1	0.24	1.2	0.02	[27]	1644	1644	2.6	0.07	1.5	0.08	[28]
20	geranyl acetone	1451	1453	0.5	0.03	-	-	[27]	1843	1840	0.9	0.19	-	-	[40]
21	*cis*-cadina-1(6),4-diene	1455	1461	0.6	0.02	0.7	0.01	[27]	1616	-	0.6	0.30	0.5	0.06	§
22	unidentified (MW = 204)	1472	-	1.3	0.06	-	-	-	1663	-	1.5	0.13	-	-	-
23	**γ-muurolene**	1475	1478	22.1	1.34	30.5	0.68	[27]	1685	1685	22.4	1.33	31.2	1.01	[36]
24	unidentified (MW = 204)	1479	-	0.3	0.01	-	-	-	-	-	-	-	-	-	-
25	*trans*-muurola-4(14),5-diene	1486	1493	0.3	0.01	0.5	0.02	[27]	-	-	-	-	-	-	-
26	**bicyclogermacrene**	1491	1500	7.7	0.53	8.9	0.21	[27]	1709	1707	7.5	0.41	7.3	0.25	[34]
27	α-muurolene	1496	1500	0.6	0.02	2.3	0.06	[27]	1702	1700	1.2	0.04	2.0	0.09	[41]
28	β-bisabolene	1501	1505	1.3	0.10	-	-	[27]	1707	1708	o.t.p. 26	-	-	-	[42]
29	γ-cadinene	1504	1513	-	-	2.4	0.08	[27]	1726	1722	-	-	2.8	0.12	[43]
30	α-alaskene	1511	1512	-	-	0.8	0.08	[27]	1749	1748	-	-	0.6	0.04	[44]
31	*trans*-calamenene	1513	1521	2.8	0.22	-	-	[27]	1808	1808	0.8	0.09	-	-	[45]
32	δ-cadinene	1515	1522			5.2	0.09	[27]	1737	1737	2.3	0.19	5.4	0.22	[46]

Table 1. *Cont.*

No.	Compounds	5%-Phenyl Methyl Polysiloxane							Polyethylene Glycol						
		LRI		*P. peltatum*		*P. umbellatum*		Literature	LRI		*P. peltatum*		*P. umbellatum*		Literature
		Calc.	Ref.	%	σ	%	σ		Calc.	Ref.	%	σ	%	σ	
33	*trans*-cadina-1,4-diene	1524	1533	0.4	0.01	0.3	0.04	[27]	1758	-	0.3	0.02	0.3	0.03	§
34	α-cadinene	1529	1537	0.2	0.01	0.3	0.02	[27]	1768	-	-	-	0.3	0.02	§
35	α-calacorene	1534	1544	0.2	0.01	0.8	0.04	[27]	1890	1887	0.6	0.08	0.8	0.01	[47]
36	β-calacorene	1556	1564	0.7	0.06	0.9	0.08	[27]	1933	1942	0.3	0.11	0.4	0.01	[48]
37	**(*E*)-nerolidol**	1561	1561	4.0	0.12	-	-	[27]	2038	2038	3.8	0.28	-	-	[49]
38	unidentified (MW = 222)	1568	-	-	-	1.2	0.05	-	2103	-	-	-	1.5	0.40	-
39	spathulenol	1569	1577	1.4	0.09	-	-	[27]	2109	2109	1.0	0.11	-	-	[50]
40	**caryophyllene oxide**	1575	1582	4.8	0.28	-	-	[27]	1957	1957	4.5	0.35	-	-	[51]
41	unidentified (MW = 222)	1576	-	-	-	1.2	0.05	-	1956	-	-	-	1.1	0.03	-
42	khusimone	1595	1604	0.3	0.02	-	-	[27]	2178	2175	0.4	0.04	-	-	[52]
43	unidentified (MW = 222)	1602	-	0.7	0.06	-	-	[27]	2013	-	0.6	0.04	-	-	-
44	junenol	1609	1618	-	-	0.4	0.02	[27]	-	-	-	-	-	-	-
45	α-corocalene	1618	1622	-	-	0.5	0.01	[27]	2037	2037	-	-	0.6	0.05	[53]
46	1,10-di-*epi*-cubenol	1618	1618	0.4	0.03	0.3	0.01	[27]	2047	2054	0.4	0.17	0.5	0.02	[54]
47	1-*epi*-cubenol	1623	1627	-	-	0.7	0.02	[27]	2046	2047	-	-	0.6	0.05	[51]
48	*epi*-α-cadinol	1632	1638	0.7	0.06	1.3	0.04	[27]	2156	2152	0.4	0.05	1.0	0.18	[47]
49	cubenol	1638	1645	0.4	0.03	-	-	[27]	2060	2059	0.5	0.11	-	-	[55]
50	α-muurolol (=torreyol)	1641	1644	-	-	0.6	0.02	[27]	2187	2187	-	-	0.6	0.06	[56]
51	unidentified (MW = 222)	1646	-	0.9	0.11	-	-	[27]	-	-	-	-	-	-	-
52	*epi*-α-muurolol	1649	1640	-	-	1.4	0.04	[27]	2217	2209	-	-	1.3	0.08	[48]
53	14-hydroxy-9-*epi*-(*E*)-caryophyllene	1662	1668	0.5	0.06	-	-	[27]	2118	-	0.3	0.08	-	-	§
54	cadalene	1666	1675	0.4	0.03	0.2	0.01	[27]	2194	2200	0.6	0.03	0.6	0.07	[36]
55	*n*-octadecanol	2081	2077	0.9	0.06	-	-	[27]	2423	-	1.2	0.17	-	-	§
56	***n*-heneicosane**	2100	2100	8.8	0.73	1.7	0.05	[27]	2100	2100	8.7	1.19	2.0	0.19	-
	monoterpenes			-		9.3					-		9.0		
	oxygenated monoterpenoids			0.5		1.5					0.9		1.5		
	sesquiterpenes			75.2		80.1					75.3		78.2		
	oxygenated sesquiterpenoids			14.1		7.0					12.0		6.5		
	others			9.7		1.7					9.9		2.0		
	total			99.5		99.6					98.1		97.1		

LRI—linear retention index; Calc.—calculated LRI; Ref.—reference LRI; Literature—the reference literature; %—percent by weight of EO; σ—standard deviation; §—identification by MS only; trace—<0.1%; o.t.p.—overlapped to peak.

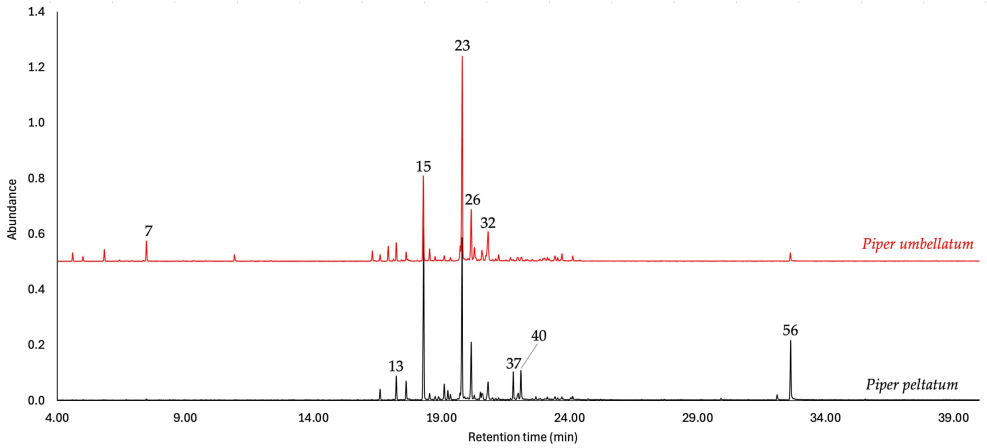


Figure 1. Comparative GC–MS profiles of *P. peltatum* (black) and *P. umbellatum* (red) EOs using the nonpolar stationary phase. Peak numbers correspond to the major constituents (≥3.0% in at least one oil and one column) listed in Table 1.

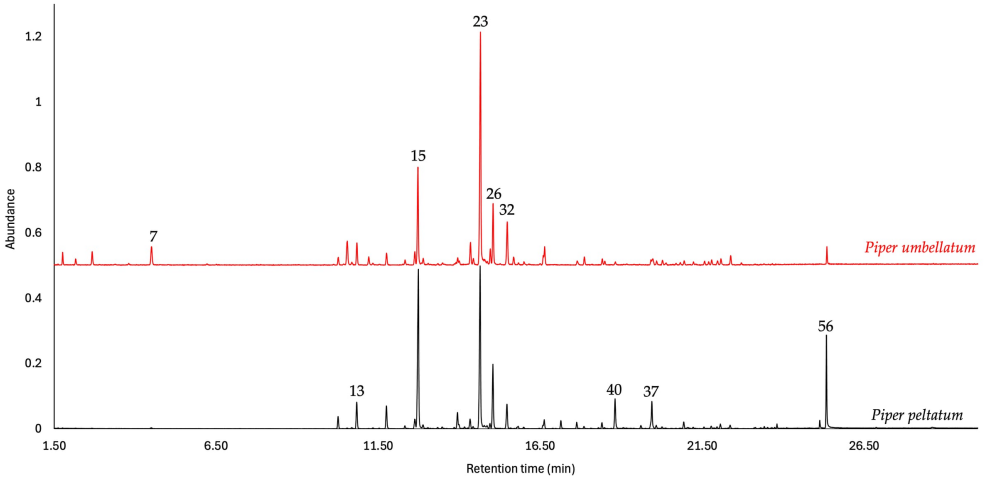


Figure 2. Comparative GC–MS profiles of *P. peltatum* (black) and *P. umbellatum* (red) EOs using the polar stationary phase. Peak numbers correspond to the major constituents (≥3.0% in at least one oil and one column) listed in Table 1.

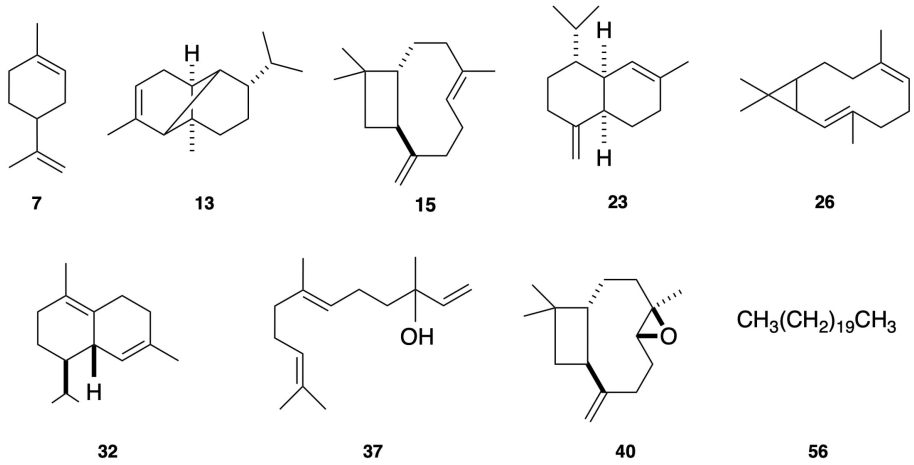


Figure 3. Chemical structures of the major constituents (≥3.0% in at least one species and one column) identified in the EOs of *P. peltatum* and *P. umbellatum*: (**7**) limonene, (**13**) α-copaene, (**15**) (*E*)-β-caryophyllene, (**23**) γ-muurolene, (**26**) bicyclogermacrene, (**32**) δ-cadinene, (**37**) (*E*)-nerolidol, (**40**) caryophyllene oxide, and (**56**) *n*-heneicosane.

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2.2. Enantioselective Analyses of the EOs

The enantioselective analysis was restricted to *P. umbellatum*, for which suitable enantiomerically pure reference standards were available, allowing the unequivocal configuration assignment of five chiral metabolites: α-pinene, camphene, β-pinene, limonene, and camphor. The separation was performed using two capillary columns with different chiral selectors. Detailed results of the enantioselective analysis, including linear retention indices (LRIs), enantiomeric distribution, and enantiomeric excess (e.e.), are presented in Table 2. On the one hand, no chiral compound was enantiomerically pure. On the other hand, α-pinene exhibited a nearly racemic distribution, with an enantiomeric excess of only 0.8%. All other chiral metabolites were present as scalemic mixtures, showing a clear predominance of one enantiomer.

Table 2. Enantioselective analysis of chiral compounds in the EO of P. umbellatum using 2,3-di-O-methyl-6-O-tert-butyldimethylsilyl-β-cyclodextrin and 2,3-di-O-ethyl-6-O-tert-butyldimethylsilyl-β-cyclodextrin as chiral selectors.

Column	Compound	LRI Calc.	LRI Ref.	Distribution (%)	e.e. (%)
DMT	(−)-(1*S*,5*S*)-α-pinene	988	985	50.4	0.8
DMT	(+)-(1*R*,5*R*)-α-pinene	996	993	49.6	
DET	(−)-(1*S*,4*R*)-camphene	919	917	8.0	84.0
DET	(+)-(1*R*,4*S*)-camphene	934	932	92.0	
DET	(+)-(1*R*,5*R*)-β-pinene	947	944	21.6	56.8
DET	(−)-(1*S*,5*S*)-β-pinene	957	955	78.4	
DET	(−)-(*S*)-limonene	1058	1056	19.4	61.2
DET	(+)-(*R*)-limonene	1074	1072	80.6	
DET	(−)-(1*S*,4*S*)-camphor	1137	1133	4.7	90.6
DET	(+)-(1*R*,4*R*)-camphor	1146	1141	95.3	

LRI—linear retention index; Calc.—calculated; Ref.—reference; %—peak area percent; e.e.—enantiomeric excess; DMT—2,3-di-O-methyl-6-O-*tert*-butyldimethylsilyl-β-cyclodextrin; DET—2,3-di-O-ethyl-6-O-*tert*-butyldimethylsilyl-β-cyclodextrin.

3. Discussion

The chemical compositions of *P. peltatum* and *P. umbellatum* EOs are compared in Figure 4, which shows the major constituents (≥3.0% in at least one EO) using the mean values obtained from both chromatographic columns. As can be observed, 5 of 9 major components are present in comparable amounts, although the relative abundances of (*E*)-β-caryophyllene and γ-muurolene are inverted.

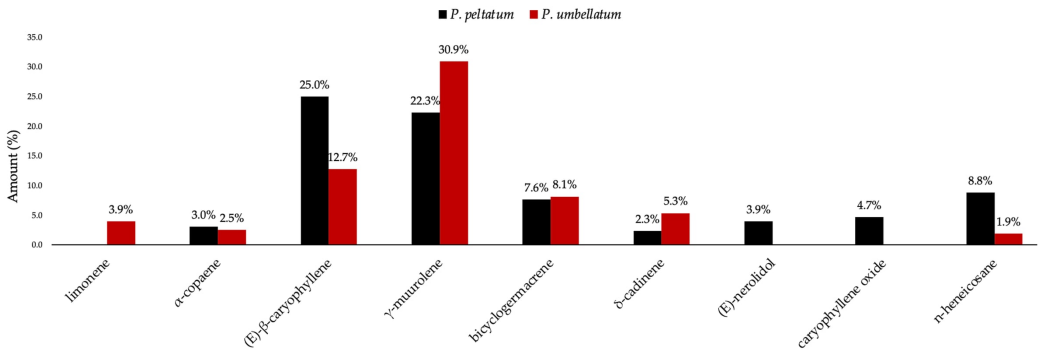


Figure 4. Comparison of the major constituents (≥3.0% in at least one species and one column) of *P. peltatum* and *P. umbellatum*. Relative abundances represent the mean quantitative values obtained using the two chromatographic columns.

The predominance of sesquiterpenes observed in both EOs was consistent with previous studies in which the sesquiterpene hydrocarbon content ranged from 31.5 to 87.7% [57,58].

Comparing the EO composition of Ecuadorian *P. peltatum* with that from other countries, it can be observed that the former is characterized by a high content of (*E*)-β-caryophyllene and γ-muurolene (Figure 5). In comparison, a Brazilian sample was characterized by (*E*)-β-caryophyllene (39.0%) and (*E*)-nerolidol (11.7%) [59], whereas caryophyllene oxide predominated in samples from Cuba (22.9%) [60] and Colombia (50.6%) [22].

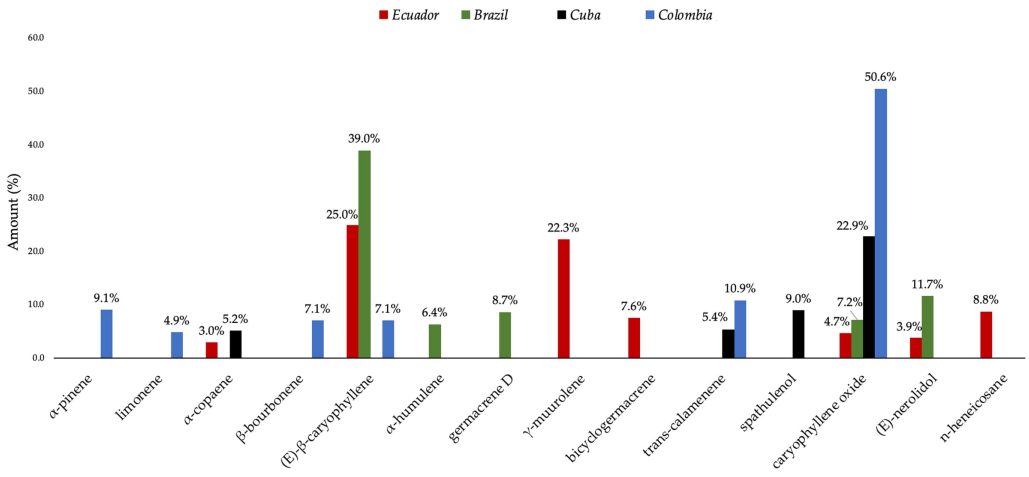


Figure 5. Comparison of the major constituents of the EO of *P. peltatum* from the Ecuadorian Amazon with those reported for samples from Brazil, Cuba, and Colombia.

The contrasting profiles illustrated in Figure 5 are consistent with the compositional diversity previously documented within the genus *Piper* [11] and underscore the value of characterizing the specific chemical profile of the Ecuadorian *P. peltatum* EO.

Furthermore, the γ-muurolene-rich profile characterized in the present study complements previously reported compositions dominated by (*E*)-β-caryophyllene or caryophyllene oxide [22,59,60].

The chemical profile of *P. umbellatum* EO was compared with previously reported compositions from Brazil [61], Cuba [62], and Costa Rica [63] (Figure 6), considering constituents with a relative abundance of ≥3.0% in at least one EO.

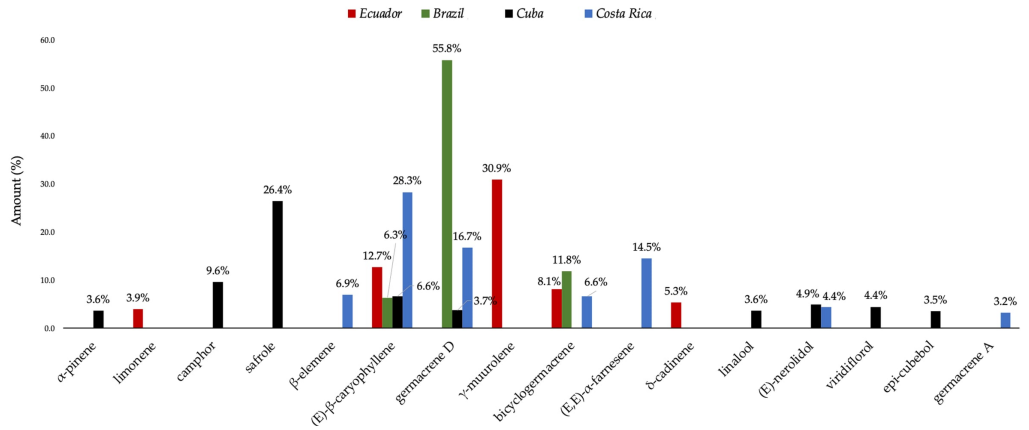


Figure 6. Comparison of the major constituents (≥3.0% in at least one EO) of *P. umbellatum* from Ecuador, Brazil, Cuba, and Costa Rica.

The *P. umbellatum* EO analyzed in the present study was characterized by γ-muurolene (30.9%) and (*E*)-β-caryophyllene (12.7%), unlike previously reported profiles that were

dominated by germacrene D in Brazil (55.8%) [61], safrole in Cuba (26.4%) [62], and (*E*)-β-caryophyllene in Costa Rica (28.3%) [63]. These compositional differences should be interpreted considering the multiple factors that may influence EO composition, including plant physiology, environmental conditions, postharvest processing, and extraction procedures [64].

The occurrence of markedly different chemical profiles within the same botanical species highlights the relevance of possible chemotypic variability in these species. However, if the existence of different chemotypes must be demonstrated, the variability in the chemical profiles of these EOs provides evidence. In this context, the essential oils characterized in the present study represent distinct chemical profiles compared with those previously reported for *P. peltatum* and *P. umbellatum*. In particular, the γ-muurolene-rich profile of Ecuadorian *P. peltatum*, characterized by the concomitant presence of substantial amounts of (*E*)-β-caryophyllene and bicyclogermacrene, differed from the caryophyllene oxide-dominated profiles reported in other geographical regions. Similarly, the predominance of γ-muurolene in *P. umbellatum* from the Ecuadorian Amazon contrasted with the germacrene D-, safrole-, and (*E*)-β-caryophyllene-dominated profiles previously described for specimens from Brazil, Cuba, and Costa Rica, respectively. These differences suggest that both species possess considerable intraspecific chemical variability, which may be associated with differences in the regulation of terpenoid biosynthetic pathways under distinct physiological and environmental conditions.

The identification of different chemical profiles is relevant not only from a phytochemical and chemotaxonomic perspective but also from an applied standpoint. Indeed, the relative abundance of specific terpenes may substantially influence the biological properties, aroma characteristics, and potential industrial applications of EOs. Consequently, specimens belonging to the same botanical species but characterized by different chemical profiles should not necessarily be considered interchangeable sources of essential oils. Instead, their individual chemical fingerprints should be taken into account when selecting plant material for specific applications, particularly in the development of standardized products for cosmetic, cosmeceutical, or other bio-based industries. In this regard, the γ-muurolene-rich profiles described herein represent additional chemical variants of two widely distributed neotropical species and expand the range of potentially exploitable volatile metabolites within the genus *Piper*.

Finally, the enantioselective characterization was restricted to *P. umbellatum* because the corresponding enantiomerically pure standards required for unequivocal configuration assignments were unavailable for most of the chiral constituents of *P. peltatum*.

Previous enantioselective studies on *P. coruscans* [26], *P. subscutatum* [65], *P. carpunya* [66], and *P. claussenianum* [67] provide a comparative context for the present findings. In particular, the shared analysis of α-pinene, β-pinene, and limonene allows the enantiomeric profiles of *P. umbellatum*, *P. coruscans*, and *P. subscutatum* to be directly compared (Figure 7). The comparison revealed differences in the predominant enantiomers and their relative enrichment [26,65]. These patterns may reflect the stereoselective action of monoterpene synthases, which can favour the formation of specific α- and β-pinene enantiomers [68,69], as well as different enantiomeric forms of limonene [70,71]. The distinct enantiomeric distributions observed in *P. umbellatum* are consistent with the stereoselective nature of monoterpene biosynthesis [72].

Several major constituents identified in the EOs of *P. peltatum* and *P. umbellatum* have exhibited documented biological activities. Limonene (**7**) has been associated with anti-inflammatory, antioxidant, antinociceptive, and anticancer properties [73], while α-copaene (**13**) has exhibited antimicrobial and antioxidant activities as well as attractant capacities for certain fruit-associated insects [74–76]. (*E*)-β-Caryophyllene (**15**) has shown anti-

inflammatory, antioxidant, immunomodulatory, and analgesic effects, partly associated with CB2 receptor activation [77,78]. γ-Muurolene (**23**) has demonstrated antioxidant, anti-inflammatory, antinociceptive [79], and antifungal activities [80]. Bicyclogermacrene (**26**) exhibits anti-inflammatory and antihyperalgesic effects [81] and has been identified as a major constituent of *Piper* EOs with antibacterial and antifungal activity [82]. δ-Cadinene (**32**) has shown antimicrobial and immunomodulatory properties, including effects on neutrophil activation [83,84]. (*E*)-Nerolidol (**37**) exhibited antimicrobial, antibiofilm, antioxidant, anti-inflammatory, antiparasitic, and insecticidal activities [85], whereas caryophyllene oxide (**40**) has demonstrated analgesic, anti-inflammatory, antimicrobial, and cytotoxic effects [86]. These findings highlight the biological relevance of the identified metabolites, although the activities of the EOs analyzed in this study were not experimentally evaluated.

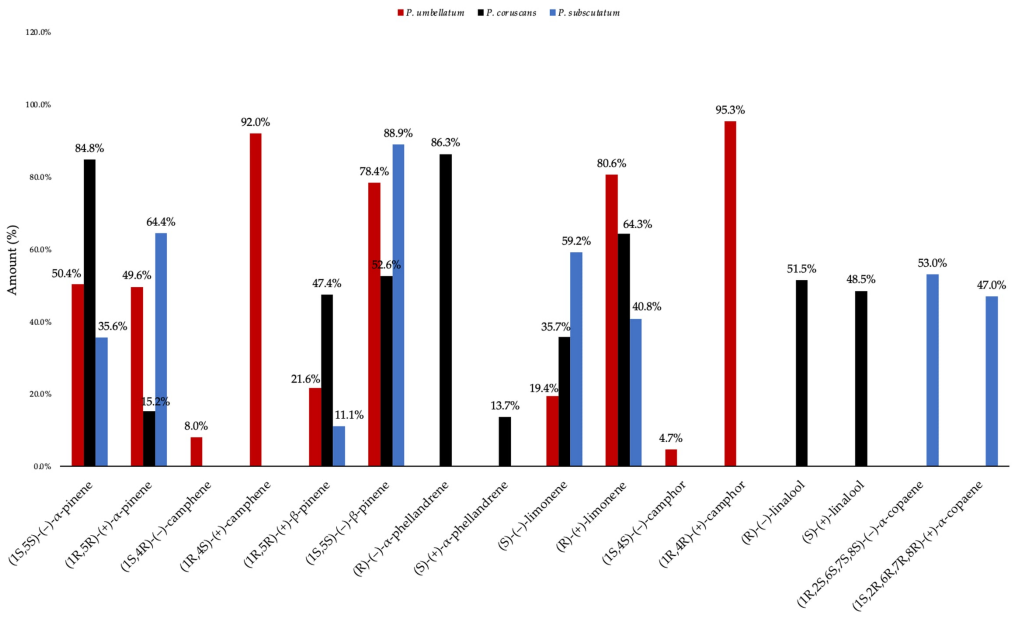


Figure 7. Comparison of the enantiomeric distribution of chiral compounds in the EOs of *P. umbellatum*, *P. coruscans*, and *P. subscutatum*.

The enantiomeric composition can influence the biological activity of chiral metabolites through stereospecific interactions with biological targets [87]. For instance, (+)-α-pinene and (+)-β-pinene have shown greater antimicrobial activity against Gram-positive bacteria and phytopathogenic fungi than their corresponding laevorotatory forms [88,89]. Similarly, (−)-limonene exhibited stronger antibacterial and antibiofilm effects against Aeromonas hydrophila than (+)-limonene [90]. These findings underline the relevance of the enantioselective characterization of *P. umbellatum*, while further experimental studies are required to establish the biological implications of its stereochemical profile. In addition to the different biological activities of the enantiomers, some biosynthetic aspects should also be considered. For instance, it can be observed that the enantiomeric excesses of α-pinene and β-pinene were quite different. This phenomenon, indeed, observed in other EOs, is remarkable because both pinenes share a common biosynthetic pathway, deriving from the same pinyl ion [91]. This carbocation already presents the two stereogenic centres of α- and β-pinene, which should be inherited unchanged by both monoterpenes. The fact that very different enantiomeric excesses were observed suggests that some stereospecific post-synthetic reaction occurred on at least one of these hydrocarbons, differently modifying the enantiomeric distribution. Another issue related to the profile of chiral compounds in EOs is the possible partial racemization produced by hot water and natural acidic conditions during steam distillation. This phenomenon especially occurs with chiral alcohols, such as

linalool and terpinene-4-ol, but it has also been observed for unsaturated hydrocarbons like limonene and, to a minor extent, pinenes [92–94]. This evidence obliges chemists to tread carefully while drawing conclusions about biochemistry based on EO compositions, since these volatile fractions are actually laboratory products rather than mixtures that precisely represent the real plant metabolic profile.

4. Materials and Methods

4.1. Plant Material

Leaves of *P. peltatum* and *P. umbellatum* were collected in the Ecuadorian Amazon, within the Witawaya and Jatari communities, respectively. Both sites are located in Pastaza Province, Ecuador. *P. peltatum* was collected on 7 November 2025 at the coordinates 1°25′58″ S and 77°41′37″ W, whereas *P. umbellatum* was collected on 10 January 2025 at the coordinates 1°38′00.05″ S and 77°57′05.84″ W. The leaves were harvested from 4 to 6 different plants within a range of about 200 m around the coordinate points, yielding two average pooled samples of plant materials, one for each species. The botanical identification of both species was performed by two of the authors (W.A.P. and F.T.). Voucher specimens were deposited in the Herbarium of the Universidad Técnica Particular de Loja (UTPL) under accession numbers HUTPL 15497 for *P. peltatum* and HUTPL 15478 for *P. umbellatum*. Plant collection was carried out under the authorization MAATE-CMARG-2022-0593-ACT-002, issued by the Ministry of the Environment, Water, and Ecological Transition of Ecuador (MAATE). After collection, the leaves of both species were dried at 35 °C for 48 h and subsequently stored in a cool, dark and ventilated place until distillation.

4.2. Distillation and Sample Preparation

The two pooled dry leaf samples of *P. peltatum* and *P. umbellatum* were separately distilled using a modified Dean–Stark apparatus, following a procedure previously described in the literature [95]. For each plant sample, one EO was obtained, representative of the different plant specimens from which the leaves were collected. A total of 65 g of dried leaves of *P. peltatum* and 221 g of dried leaves of *P. umbellatum* were distilled for 4 h, until a constant volume of collected oil was obtained. After distillation, the EOs obtained from both species were stored at −15 °C until use.

In sample preparation, four independent analytical replicate solutions were prepared from the EOs of each species. For each replicate, a 5 µL aliquot of EO was individually weighed. To each aliquot, 1 mL of analytical-grade cyclohexane, containing *n*-nonane as an internal standard, was added. The internal-standard solution was 0.7 mg/mL. Cyclohexane and *n*-nonane were obtained from Merck (Sigma-Aldrich, St. Louis, MO, USA). The prepared solutions were subsequently directly used for gas chromatographic (GC) analyses.

4.3. Qualitative (GC-MS) Chemical Analyses

The qualitative analyses of the EOs of *P. peltatum* and *P. umbellatum* were performed using a model 8890 gas chromatograph coupled to a 5977C single-quadrupole mass spectrometer, both supplied by Agilent Technologies (Santa Clara, CA, USA). Chromatographic separations were carried out using two capillary columns coated with stationary phases of different polarities: 5% phenyl methyl polysiloxane (TR-5ms, nonpolar) and polyethylene glycol (TR-WAX, polar). Both columns, supplied by Thermo Fisher Scientific (Waltham, MA, USA), measured 20 m × 0.18 mm i.d. × 0.18 µm film thickness. High-purity hydrogen (99.9995%), generated by a Peak Scientific electrolytic generator (Glasgow, UK), was used as the carrier gas at a constant flow rate of 0.8 mL·min^{-1}.

For the nonpolar phase, the oven temperature was initially set at 50 °C and maintained for 4 min. The temperature was then increased at 5 °C·min^{-1} to 220 °C and maintained

for 5 min. For the polar phase, the initial temperature was 50 °C, maintained for 4 min, followed by an increase of 7 °C·min^{-1} to 180 °C. A second temperature ramp of 34 °C·min^{-1} was then applied up to 220 °C, which was maintained for 6 min.

For both chromatographic methods, 0.5 µL of sample was injected in split mode, using a split ratio of 40:1, while the injector temperature was maintained at 220 °C. The mass spectrometer was operated in electron-impact ionization mode (EIMS) at 70 eV. The ion source and quadrupole temperatures were maintained at 230 and 150 °C, respectively, while the transfer line was set at 220 °C. Mass spectra were acquired in SCAN mode over a mass range of 40–400 m/z.

The EO constituents were identified by comparing their EIMS and experimental linear retention indices (LRIs) with data reported in the literature. LRIs were calculated according to Van den Dool and Kratz [96], using a homologous series of *n*-alkanes in the range C_9–C_{22} analyzed under the same chromatographic conditions. The alkane standards were supplied by Merck (Sigma-Aldrich, St. Louis, MO, USA).

4.4. Quantitative (GC-FID) Chemical Analyses

The quantitative determination of the EO components was performed using the same GC system previously described, coupled to a flame ionization detector (GC–FID). The injector temperature was maintained at 220 °C, whereas the oven thermal programmes were the same as in the GC-MS analysis. High-purity hydrogen (99.9995%), generated by the same Peak Scientific electrolytic generator used to supply the carrier gas, was employed to sustain the detector flame. High-purity air (99.9995%), supplied from a cylinder by Indura S.A. (Guayaquil, Ecuador), was used as the comburent. Hydrogen and air were delivered at flow rates of 30 and 300 mL·min^{-1}, respectively. Nitrogen (99.9995% purity), supplied from a cylinder by the same company, was used as the make-up gas, at a flow rate of 25 mL·min^{-1}.

For quantification, the chromatographic areas of each analyte were corrected using relative response factors (RRFs), calculated based on their respective enthalpies of combustion, following the model developed by A. Chaintreau [97,98]. For all unidentified compounds, the molecular formulas were deduced from the mass of the molecular ion, assuming that they were isomers of known metabolites with the same molecular mass. The corrected values were subsequently applied to calibration curves, constructed at six concentration levels. *n*-Nonane was used as the internal standard, whereas isopropyl caproate was employed as the calibration standard. The resulting calibration curves exhibited coefficients of determination (R^2) greater than 0.999, with the calibration solutions prepared according to previously reported procedures [26]. The internal standard was provided by Merck (Sigma-Aldrich, St. Louis, MO, USA), whereas the calibration standard was synthesised in the authors' laboratory and purified to 98.8% (GC-FID purity).

4.5. Enantioselective Analyses

The enantioselective analysis of the EO of *P. umbellatum* was performed by GC–MS, using the same instrument described for the qualitative analysis. Enantiomer separation was achieved using two capillary columns coated with β-cyclodextrin-derived chiral stationary phases: 2,3-di-O-methyl-6-O-*tert*-butyldimethylsilyl-β-cyclodextrin (DMT) and 2,3-di-O-ethyl-6-O-*tert*-butyldimethylsilyl-β-cyclodextrin (DET), both supplied by Mega S.r.l. (Legnano, Italy). The columns were 25 m long, with an internal diameter of 0.25 mm and a film thickness of 0.25 µm.

Helium was used as the carrier gas at the constant flow rate of 1 mL·min^{-1} (Indura S.A., Guayaquil, Ecuador). The oven temperature was initially maintained at 50 °C for 5 min and then increased at 2 °C·min^{-1} to 200 °C, which was held for 15 min. The injector and MS transfer line were maintained at 220 °C, while the ion source was set at 230 °C.

Electron-impact ionization was performed at 70 eV. A sample volume of 0.5 µL was injected in split mode, using a split ratio of 40:1.

The enantiomers were identified by comparing the experimentally obtained linear retention indices (LRIs) with those of enantiomerically pure standards analyzed under the same chromatographic conditions. Some of these standards were purchased from Merck (Sigma-Aldrich, St. Louis, MO, USA), while others were available at the University of Turin (Italy). Retention indices were calculated according to Van den Dool and Kratz [96], employing a homologous series of *n*-alkanes C_9–C_{22} that were purchased from Merck (Sigma-Aldrich, St. Louis, MO, USA). The enantiomeric distribution and excess were calculated based on the injection of a single sample (and therefore without standard deviation), since the preparation of different analytical replicates from the same EO is not an enantioselective process. Therefore, it cannot modify the internal enantiomeric ratio of the sample.

5. Conclusions

The leaves of *P. peltatum* and *P. umbellatum*, collected in the Ecuadorian Amazon, produced two EOs with a relatively high distillation yield. Qualitative and quantitative analyses allowed us to establish that their chemical profiles are quite different from those of EOs distilled from the same species from other sites (e.g., Brazil).

In addition, the enantioselective analysis of *P. umbellatum* allowed for the characterization of five chiral metabolites: α-pinene, camphene, β-pinene, limonene, and camphor. α-Pinene exhibited a nearly racemic distribution, while the other compounds showed varying degrees of enantiomeric enrichment, with (+)-(1*R*,4*R*)-camphor being the compound with the greatest enantiomeric excess. Taken together, these results provided new information on the chemical characterization of *P. peltatum* and *P. umbellatum* leaf EOs, constituting the first step in their selection as commercial products in a new bioeconomic activity for the indigenous communities of the Ecuadorian Pastaza province.

Although the major components observed in these oils are very common in nature, the biological properties described in the literature make them promising for a potential cosmeceutical application. Nevertheless, an experimental confirmation of their biological activities should be the subject of further studies.

Author Contributions: Conceptualization, G.G. and O.M.; investigation, K.C., W.A.P. and F.T.; data curation, K.C. and Y.E.M.; writing—original draft preparation, K.C.; writing—review and editing, K.C. and G.G.; supervision, G.G. and O.M. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The datasets generated and analyzed in this study are not publicly available, as they form part of ongoing research. However, they may be obtained from the corresponding author upon reasonable request.

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Conflicts of Interest: The authors declare no conflicts of interest.

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