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Essential oil composition of *Lithraea molleoides* (Vell.) Engler (Anacardiaceae), a controversial medicinal, edible, and allergenic species from South America

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Introduction and Aim

Lithraea molleoides (Vell.) Engler (Anacardiaceae family) is an evergreen tree species native from South America, considered in Argentina as a medicinal and edible plant [1]. The infusions/decoctions from the vegetative aerial parts are used as medicines for respiratory and digestive illnesses [1], while the fruits are employed to elaborate fermented beverages [1]. However, in Uruguay this plant is not recognized as medicinal/edible, and it is considered highly allergenic, with frequently reported cases of contact dermatitis in the face and/or arms occurring in sensibilized people [2]. Not volatile alk(en)yl-catechols (ACs) have been pointed out as responsible of such contact dermatitis [2]. Moreover, an orally transmitted tradition in Uruguay indicates that such affections occur when the people just approach to the trees, without being necessary the contact (that is, an eventual airborne allergy). This behavior suggests the intervention of volatile allergens in the process, a fact that needs to be better investigated given the ethnobotanical use of this species. As a first step to validate such traditional information, the aim of this work was to characterize the chemical compositions of *L. molleoides* essential oils of Uruguayan origin using different GC-MS methods and stationary phases.

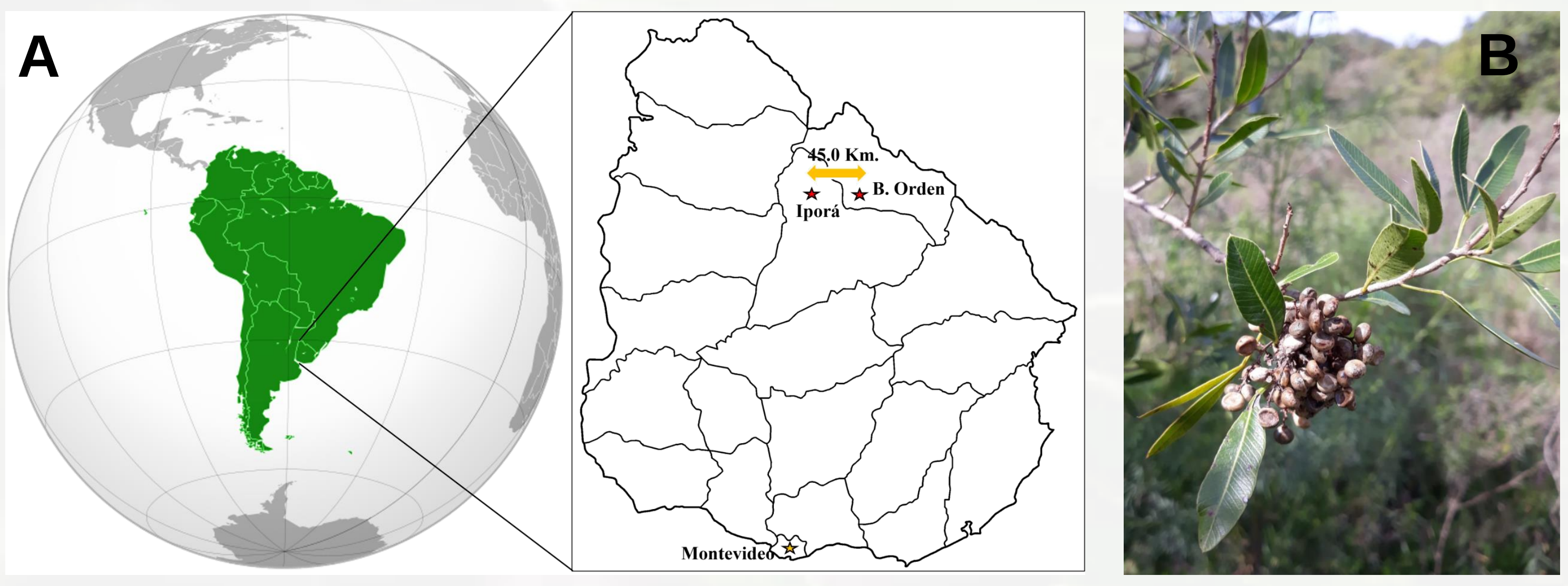


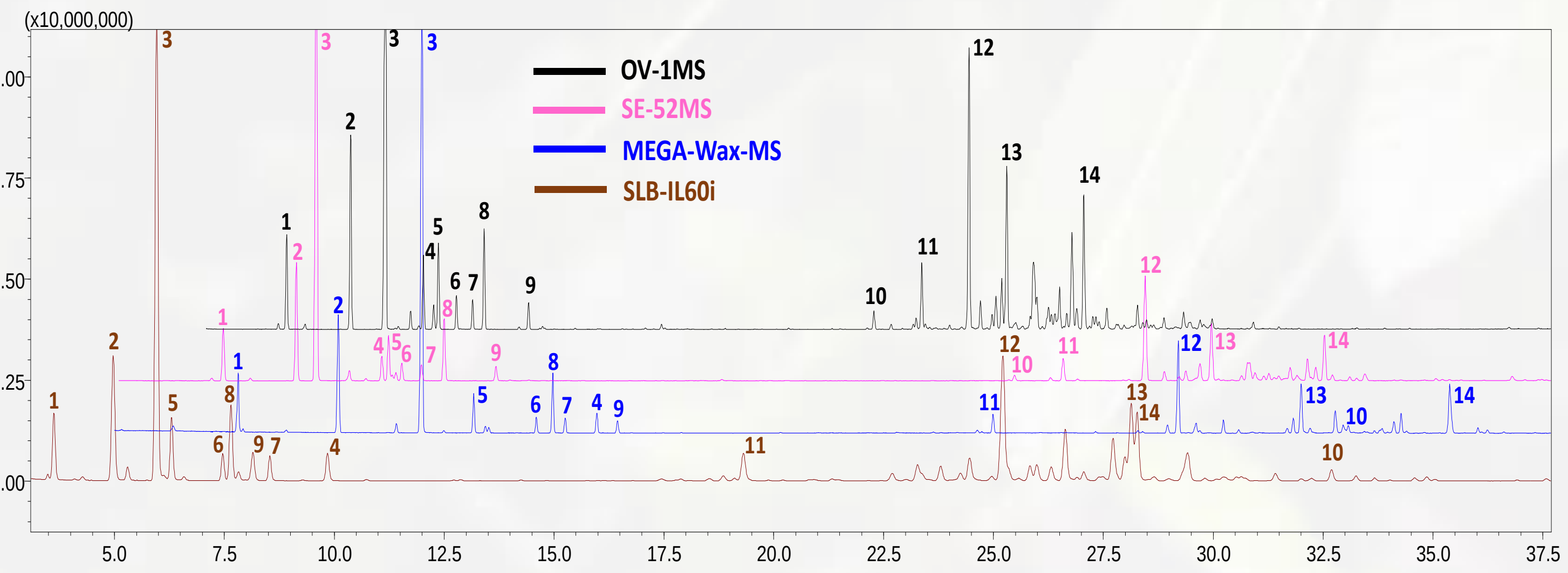
Figure 1: **(A)** Area of study of this work in the Uruguayan Northeastern region. **(B)** Aerial parts of *Lithraea molleoides* (Vell.) Engler (Anacardiaceae family): leaves and fruits that were employed to obtain the essential oil (photo: M. Minteguiaga).

Methods

L. molleoides sampling [leaves + small stems (**L**) and fruits (**F**)] were performed in the Northeastern region of Uruguay: *Iporá* (**I**) and *Buena Orden* (**BO**) locations (Tacuarembó and Rivera Provinces, respectively; Figure 1), during three different seasons (South Hemisphere): Spring 2021 (**SP21**), Spring 2022 (**SP22**) and Summer 2023 (**SM23**). Four collections were made to date: **L-BO-SP21**, **L-I-SP22**, **L-I-SM23** and **F-I-SM23**. The essential oils were obtained by both hydrodistillation at laboratory scale and steam distillation at pilot scale [yields: 0.2% (v/w)]. The oils were dried and diluted properly before the analyses. The use of different GC-MS stationary phases allowed to obtain more detailed information about the composition of the samples: OV-1MS, SE52-MS, MEGA-Wax-MS, SLB-IL60i, 2,3-*O*-diethyl-6-*O*-tertbutyldimethylsilyl-β-cyclodextrin (**CD1**), and 2,3-*O*-dimethyl-6-*O*-pentyl-β-cyclodextrin (**CD2**) using the oven programs shown in Figures 2 to 4. Mass spectra and linear retention index (***I*** $^{T}_{S}$) comparisons with commercial/in-house libraries allowed to identify the components. To corroborate the elution order of α-thujene, δ-3-carene and *trans*-nerolidol enantiomers in **CD2**, commercial essential oils of *Rosmarinus officinalis* L., *Piper nigrum* L. and *Citrus x auriantum* L. (neroli) (respectively) were analyzed in the same conditions as *L. molleoides* samples. Enantiomeric composition (EC) was determined by integration of reference ions in EI analysis mode.

Results and Discussion

For a better understanding of the chromatographic behavior of the samples analyzed on the different stationary phases, **L-I-SM23** was adopted as a reference (Figure 2). As expected, selectivity differences were evidenced for each phase [e.g. in Figure 2 the case of *p*-cymene (**4**) and terpinyl acetate (**10**)]. Based on the chromatographic efficiency and the availability of information to properly identify the components of the essential oils, SE-52MS was selected to compare the samples (Figure 3).



Oven temperature programs:

OV-1MS: 40°C (5min)-5°Cmin^{-1}-235°C (2min).

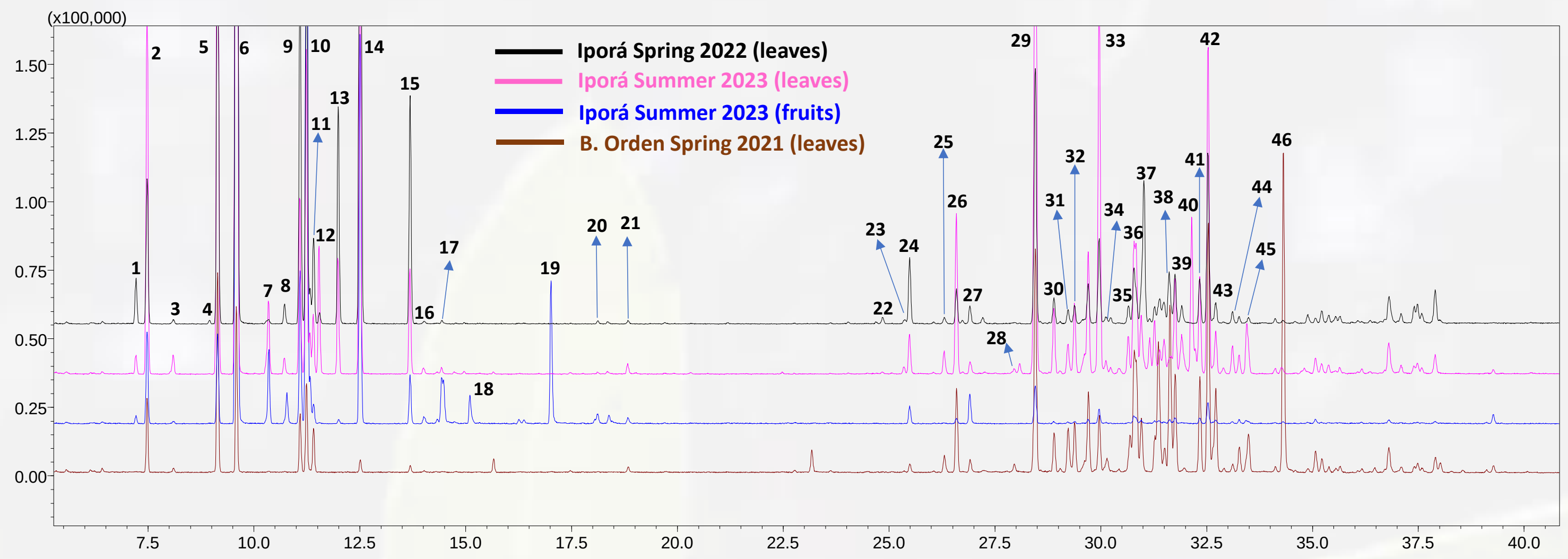
SE-52MS: 50°C (1min)-3°Cmin^{-1}-250°C (5min).

MEGA-Wax-MS: 50°C (1min)-3°Cmin^{-1}-250°C (5min).

SLB-IL60i: 50°C (1min)-2°Cmin^{-1}-230°C (5min).

Figure 2: L-I-SM23 essential oil analyzed by GC-MS (different stationary phases). Selected markers: **1.** α-pinene; **2.** β-pinene; **3.** myrcene; **4.** *p*-cymene; **5.** limonene; **6.** *cis*-β-ocimene; **7.** *trans*-β-ocimene **8.** γ-terpinene; **9.** α-terpinolene; **10.** α-terpinyl acetate; **11.** α-copaene; **12.** *trans*-β-caryophyllene; **13.** α-humulene; and **14.** δ-cadinene

Figure 3 shows the GC-MS profiles of the different samples analyzed, while a summary of their chemical composition is presented in Table 1. Monoterpene and sesquiterpene hydrocarbons were the main components identified (myrcene being the more abundant one), with at least 23 of them previously reported as contact allergy elicitors (Table 1) [3]. As expected, by their low volatility, ACs were not detected in the samples.



Oven temperature program:

SE-52MS: 50°C (1min)-3°Cmin^{-1}-250°C (5min).

Figure 3: *L. molleoides* essential oils composition analyzed by GC-MS (SE-52MS). Peak identification: **1.** α-thujene; **2.** α-pinene; **3.** camphene; **4.** sabinene; **5.** β-pinene; **6.** myrcene; **7.** δ-3-carene; **8.** α-terpinene; **9.** *p*-cymene; **10.** limonene; **11.** 1,8-cineole; **12.** *cis*-β-ocimene; **13.** *trans*-β-ocimene; **14.** γ-terpinene; **15.** α-terpinolene; **16.** *p*-cymenene; **17.** linalool; **18.** methyl *cis*-4-octenoate; **19.** *trans*-ocimenol; **20.** terpinen-4-ol; **21.** α-terpineol; **22.** δ-elemene; **23.** α-cubebene; **24.** terpinyl acetate; **25.** α-ylangene; **26.** α-copaene; **27.** geranyl acetate; **28.** methyleugenol; **29.** *trans*-β-caryophyllene; **30.** β-copaene; **31.** aromadendrene; **32.** guaia-6,9-diene; **33.** α-humulene; **34.** *allo*-aromadendrene; **35.** *trans*-cadina-1(6),4-diene; **36.** γ-muurolene + *epi*-bicyclosesquiphellandrene; **37.** α-amorphene; **38.** α-selinene; **39.** α-muurolene; **40.** β-bisabolene; **41.** γ-cadinene; **42.** δ-cadinene, **43.** *cis*-calamenene; **44.** cadina-1,4-diene; **45.** *trans*-α-bisabolene + α-calacorene; **46.** *trans*-nerolidol; **47.** *epi*-α-cadinol.

The enantioselective GC-MS (eGC-MS) analysis was optimized using the **L-I-SM23** essential oil sample, by employing two different chiral selectors as stationary phases (**CD1** and **CD2**). **CD2** demonstrated the best results in terms of separation power of the chiral and non-chiral analytes (Figure 4). Thus, **CD2** was selected to obtain the EC of monoterpene chiral compounds from the different samples as a genuineness and identity criteria (Table 1).

Oven temperature programs:

CD1: 50°C (0min)-2°Cmin^{-1}-220°C (2min).

CD2: 50°C (0min)-2°Cmin^{-1}-200°C (0min).

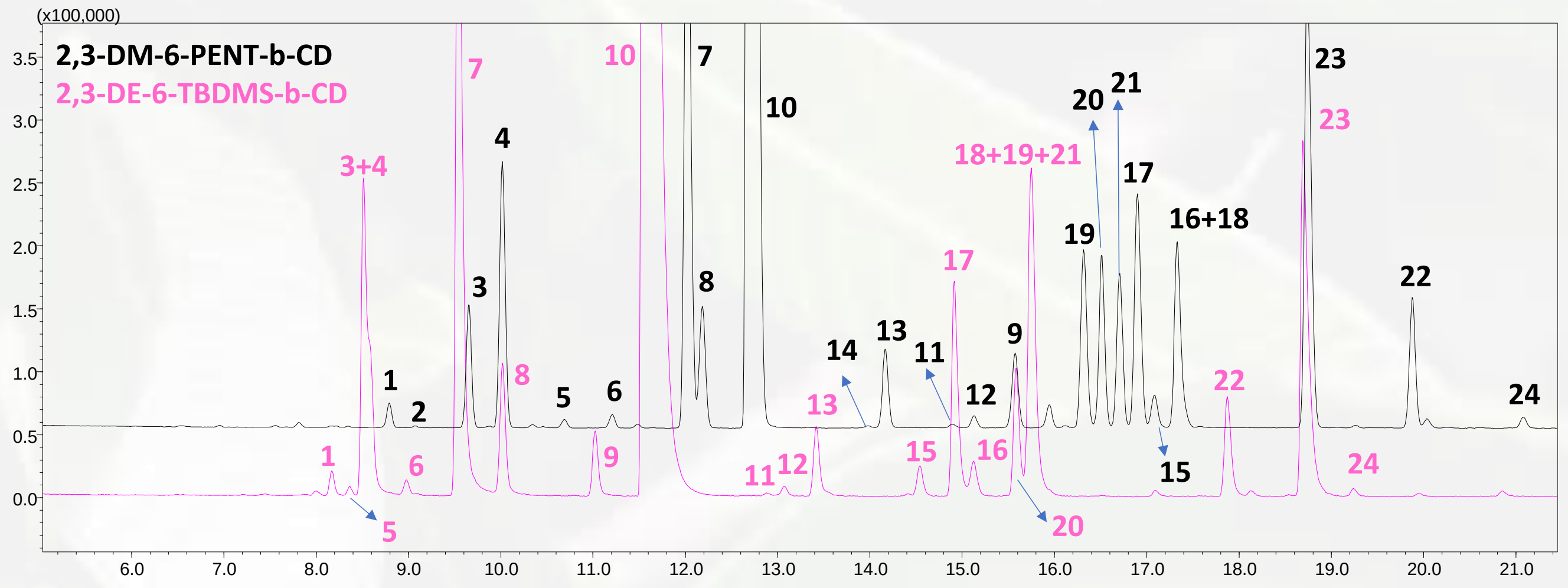


Figure 4: eGC-MS analysis of the monoterpene hydrocarbon fraction of L-I-SM23 sample with CD1 and CD2 as chiral selectors. Peak identification: **1.** (+)-α-thujene; **2.** (-)-α-thujene; **3.** (1*S*)-(-)-α-pinene; **4.** (1*R*)-(+)-α-pinene; **5.** (1*S*,4*R*)-(-)-camphene; **6.** (1*R*,4*S*)-(+)-camphene; **7.** (1*R*)-(+)-β-pinene; **8.** (1*S*)-(-)-β-pinene; **9.** 1,8-cineole; **10.** myrcene; **11.** (*R*)-(-)-α-phellandrene; **12.** (*S*)-(+)-α-phellandrene; **13.** (-)-δ-3-carene; **14.** (+)-δ-3-carene (not detected with CD1); **15.** (-)-β-phellandrene; **16.** (+)-β-phellandrene; **17.** (*S*)-limonene; **18.** (*R*)-limonene; **19.** *p*-cymene; **20.** *cis*-β-ocimene; **21.** *trans*-β-ocimene; **22.** α-terpinolene; **23.** γ-terpinene; **24.** *p*-cymenene.

	(I^{T}_{S}) OV-1MS		(I^{T}_{S}) SE-52MS		(I^{T}_{S}) Wax-MS		(I^{T}_{S}) CD1		(I^{T}_{S}) CD2		% Enantiomeric Composition in CD2*				
Compound	**exp**	**lit^{1}**	**exp**	**lit^{2}**	**exp**	**lit^{1}**	**exp**	**lit^{3}**	**exp**	**lit^{3}**	**L-BO-SP21**	**L-I-SP22**	**L-I-SM23**	**F-I-SM23**	**Ref. ion (m/z)**
(+)-α-thujene	920	931	924	924	1037	1038	918$^{\&}$	#	913	#	nd	97.8	93.8	97.9	93
(-)-α-thujene								#	918	#		2.2	6.2	2.1	
(1*S*)-(-)-α-pinene	925	939	930	932	1032	1036	925$^{\&}$	923	930	929	20.4	53.7	31.0	28.4	93
(1*R*)-(+)-α-pinene								921	937	936	79.6	46.3	69.0	71.6	
(1*S*,4*R*)-(-)-camphene	936	952	944	946	1075	1066	921	917	950	949	33.0	41.6	38.1	43.4	93
(1*R*,4*S*)-(+)-camphene							935	932	960	959	67.0	58.4	61.9	56.6	
(1*R*,5*R*)-(+)-sabinene	961	972	972	969	1128	1130	975	972	nd	nd	nd	35.6	nd	nd	93
(1*S*,5*S*)-(-)-sabinene							988	988				65.4			
(1*R*)-(+)-β-pinene	963	978	978	974	1115	1120	947	944	975	975	87.2	91.1	86.0	78.2	93
(1*S*)-(-)-β-pinene							958	955	978	979	12.8	8.9	14.0	21.8	
(*R*)-(-)-α-phellandrene	991	1000	1003	1002	1170	1173	1018	1017	1027	1027	nd	nd	23.0	nd	93
(*S*)-(+)-α-phellandrene							1022	1020	1031	1030			77.0		
myrcene	983	986	991	988	1170	1166	-	-	-	-	nch	nch	nch	nch	-
(+)-δ-3-carene	998	1009	1008	1008	1153	1156	1018	1018	1011	1012	nd	0.0	2.6	2.0	93
(-)-δ-3-carene							1028	1027	1015	#		100.0	97.4	98.0	
α-terpinene	1003	1016	1015	1014	1184	1188	-	-	-	-	nd	nch	nch	nd	-
***p*-cymene**	1006	1020	1022	1020	1272	1272	-	-	-	-	nch	nch	nch	nch	-
(*S*)-limonene	1016	1024	1026	1024	1203	1206	1057	1056	1061	1061	17.8	74.8	57.0	15.9	68
(*R*)-limonene							1072	1072	1068	1068	82.2	25.2	43.0	84.1	
1,8-cineole	1012	1017	1028	1026	1209	1223	-	-	-	-	nch	nch	nch	nch	-
α-terpinolene	1074	1074	1086	1086	1284	1287	-	-	-	-	nch	nch	1.0	0.7	-
(*R*)-(-)-linalool	1083	1086	1100	1095	1544	1555	1180	1174	1213	1212	nd	30.0	16.8	62.5	71
(*S*)-(+)-linalool							1195	1189	1223	1222		70.0	83.2	37.5	
nonanal	1081	nr	1110	1100	nd	nr	-	-	-	-	nd	nd	nch	nch	-
(*S*)-(+)-4-Terpinen-4-ol	1154	1170	1183	1174	nd	1628	1252	1248	1319	1318	57.0	40.1	48.2	34.1	71
(*R*)-(-)-4-Terpinen-4-ol							1256	1253	1327	1326	43.0	59.9	51.8	65.9	
(*R*)-(+)-α-terpineol	1166	1178	1189	1186	1681	1685	1313	1309	1366	1366	17.8	26.5	18.0	29.4	59
(*S*)-(-)-α-terpineol							1300	1296	1370	1370	82.2	73.5	82.0	70.6	
α-terpinyl acetate	1328	1333	1348	1346	1689	1687	-	-	-	-	ns	ns	ns	ns	-
(-)-α-copaene	1367$^{\&}$	1369	1372	1374	1487	1493	1318$^{\&}$	1317	1342	1344	31.4	29.1	31.4	40.7	119
(+)-α-copaene							1320$^{\&}$	1319	1354	1355	68.6	70.9	68.3	59.3	
geranyl acetate	1360	1363	1385	1379	nd	1754	-	-	-	-	nch	nch	nch	nch	-
methyl eugenol	1367$^{\&}$	nr	1406	1403	2003	2016	-	-	-	-	nch	nd	nch	nch	-
***trans*-β-caryophyllene**	1406	1417	1414	1417	1590	1617	-	-	-	-	ns	ns	ns	ns	-
aromadendrene	1426	nr	1434	1439	1637	1650	-	-	-	-	ns	ns	ns	ns	-
ledene	1481	1484	1489	1496	nd	1697	-	-	-	-	nd	ns	ns	ns	-
(*R*)-(-)-*trans*-nerolidol	1545	1553	1561	1561	2032	2044	-	-	1649	#	96.2	22.7	nd	68.8	69
(*S*)-(+)-*trans*-nerolidol							-	-	1657	#	3.8	77.3		31.2	
caryophyllene oxide	1557	1576	1578	1587	1968	1966	-	-	-	-	ns	ns	ns	ns	-

Table 1: Summary of the main components of *L. molleoides* essential oils. In **bold** are shown the compounds cited as allergenics [3]. Literature data I^{T}_{S} from: (1) Davies (1990) [4]; (2) Adams (2017) [5]; and (3) Liberto *et al.* (2008) [6]. nd: not detected; nch: no chiral compound; ns: not separated. (*) Enantiomeric composition determined by integration of the reference ions. (#) determined after analysis of *R. officinalis*, *P. nigrum* and Neroli essential oils. (&) co-elutions.

In this work were evidenced important differences in EC between the samples, as shown in Table 1. This fact needs to be better studied through the analysis of more samples collected in different locations and different seasons. Furthermore, a reliable quantification method based on GC-FID (internal standard) would be applied to generate more information about authenticity.

Conclusion

The application of different GC-MS approaches demonstrated the complexity of *L. molleoides* essential oils. At least 23 reported volatile allergens were detected as main components in the samples and a high EC variation pattern was observed between them, suggesting the need of more detailed investigations to ensure the safe use of this plant species and to assure the genuineness.

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