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Chemical and Olfactory Analysis of the Volatile Fraction of *Ocimum gratissimum* Concrete from Madagascar

Laure Gilles^[a, b] and Sylvain Antoniotti^{*[a]}

The chemical composition of the volatile fraction of *Ocimum gratissimum* concrete (romba) from Madagascar has been determined for the first time by GC/MS and GC-FID. A methyl cinnamate chemotype has been determined for this material, along with a set of compounds typical in essential oils and extracts from plants of the *Ocimum* genus. Variability was mostly observed on terpenes and terpenoids components. GC-O-MS was also used for a sensory evaluation of this material

performed by a master perfumer. The chemical composition of this *O. gratissimum* extract was then compared with literature data to assess subtle differences between chemotypes of the same species and other species of the same genus within natural variability. A mapping illustrates the occurrence of the cinnamate chemotype in Eastern Africa, India and now Madagascar, while other origins generally present eugenol, thymol, camphor, or linalool chemotypes.

Introduction

Ocimum gratissimum or clove basil, is a plant of the Lamiaceae family (Table 1, Figure 1) which is commonly found in Africa, Asia and South America. The use of its leaves as an infusion has been reported in folk medicine to treat fever, flu and kidney problems.^[1]

Many studies have been conducted on *Ocimum gratissimum*, including the total or partial determination of the composition of its essential oil (62 publications), but almost all of these articles focused on the bioactive properties of the plant.^[1–9] Indeed it showed antibacterial,^[10] antimicrobial,^[1] acaricides,^[3] properties, often associated with the eugenol and the thymol chemotypes.^[11–13]

The established chemical compositions show that nine different chemotypes have been identified for *Ocimum gratissimum* (Table 2), although eugenol and thymol are the most common (Entries 8 and 9).^[14] The chemotype is assigned based on the chemical composition of which a predominant chemical entity can be identified, and can vary within the same species according to their ecosystem. It depends essentially on the growing region of the plant and the climate (altitude, humidity, biotope, sun exposure, temperature, rain) since the influence of the ecosystem can cause chemical variations in the secondary metabolites of the plant.

Table 1. Classification of *Ocimum gratissimum*.

Plant Kingdom	
Class	Equisetopsida
Sub-class	Magnoliidae
Super order	Asteranae
Order	Lamiales
Family	Lamiaceae
Genus	<i>Ocimum</i>
Species	<i>gratissimum</i>



Figure 1. *Ocimum gratissimum* (picture: Rison Thumboor from Thrissur, India).

Table 2. Chemotypes for *Ocimum gratissimum*.^[14]

Entry	Chemotypes
1	Linalool/estragole
2	Eugenol/1,8-cineole/sesquiterpenes
3	Methyl cinnamate
4	Methyleugenol/eugenol
5	Ethyl cinnamate
6	Citral
7	Geraniol
8	Eugenol
9	Thymol

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Regarding the olfactory evaluation of the essential oil, out of a hundred publications reporting studies of the plant, only two have been interested in describing olfactory properties. The former dealt with the overall olfactory evaluation of the essential oil to confirm the chemotype found by studying the composition by GC/MS and GC-FID.^[15] The latter carried out GC-O experiments on the essential oils of 4 species of the *Ocimum* genus including *Ocimum gratissimum*.^[16] This article, written in Mandarin, reported a work carried out for the determination of the composition of the essential oil by GC/MS, and the use of GC-O analysis in order to confirm the olfactory profile of the compounds identified in GC/MS, albeit in the absence of calculations of the Kovats retention indices (RI), essential for a correct characterization in addition to the comparison of mass spectra and coinjection of authentic samples in case of doubt.

In addition, the diversity of the types of extracts on which *Ocimum gratissimum* studies have been performed is quite low. Almost all of them focused on essential oil, except for two articles on the analysis of a solvent extract. The first focused on a freeze-dried ethanolic extract, somehow devoid of volatile molecule.^[17] In the second, a methanol extraction was carried out, then, after evaporation, the residue was dissolved in distilled water and liquid-liquid extractions with hexane, diethyl ether and ethyl acetate were carried out.^[18] The fractions obtained were purified on preparative HPLC to perform biological testing. Finally, methyl cinnamate was isolated and found to be responsible for insecticidal and larvicidal activities.

Herein, we wish to describe the work carried out in the frame of global project involving a Master perfumer to identify promising odorant molecules in natural materials besides odor-impact molecules specific of the mixture. In particular, we present our results on a romba (*Ocimum gratissimum*) concrete (solvent extract) with GC-FID, GC/MS and GC-O analysis performed on the concrete and the volatile fraction of the concrete obtained after distillation at low pressure.

Discussion

Methodology

For several sample, the superposition of the FID chromatogram with the olfactogram composed of the olfactory impressions of a professional perfumer was generated. Several parameters made it possible to drive us towards the concrete of Romba (*Ocimum gratissimum*) in order to carry out a more in-depth analysis of the data obtained. The factors taken into account were the complexity of the chromatographic profile but also the interest of the perfumer for the scents felt, and on their possible response to a need of the perfumery industry.

Distillation of romba concrete (*Ocimum gratissimum*)

The romba sample used in this study was a concrete (supplied by Bionexx), consisting in a raw solvent extract made from the

leaves and stems of *Ocimum gratissimum* plants from Madagascar.

In order to focus only on the volatile part, the concrete was distilled in a Kugelrohr-type distillation oven under reduced pressure, down to 1 mbar (Figure 2).

A quantity of 53.4 g of concrete was submitted to the distillation process and 23.3 g of distillate was obtained (44% yield).

The GC/MS chromatogram of the distillate showed the same profile as the chromatogram of the concrete, which confirmed that all volatile compounds have been preserved and that distillation has not altered their relative proportions nor generated new compounds.

Distillate composition

The chemical composition of the distillate was established following standard procedures by combining two methods:

Comparison of the mass spectrum of each peak of the GC/MS chromatogram with those of the commercial library databases (NIST15, Wiley) and those of the database established by Adams.^[19]

The comparison of the Kovats retention indices (RI) determined on our mixture with those of this database.

The main components of this distillate were found to be camphor, estragole, methyl (*Z*)-cinnamate, eugenol, methyl (*E*)-cinnamate (major compound) and *trans*- α -bergamotene, indicating a cinnamate chemotype (Figure 3).

A total of 56 volatile compounds constituting 99.23% of the distillate of the romba concrete were identified (Table 3), their relative proportion was determined by GC-FID. Interestingly, metabolites of different biosynthetic origin were found with terpenes and terpenoids (including phenolics and sesquiterpenes), phenylpropanoids, and fatty acid derivatives.

Thanks to the identification of most of the compounds, the correlation between the olfactory notes felt by the perfumer and the associated odorous molecule could be established (Table 4). In addition, the organoleptic properties of these molecules have been confirmed by comparison with informa-

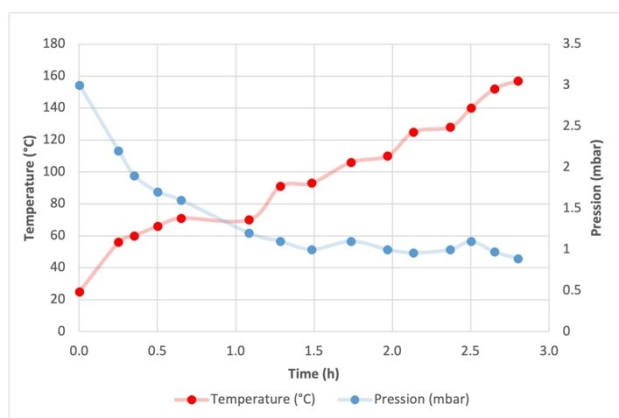


Figure 2. Monitoring of temperature and pressure during the distillation of romba concrete (*Ocimum gratissimum*).

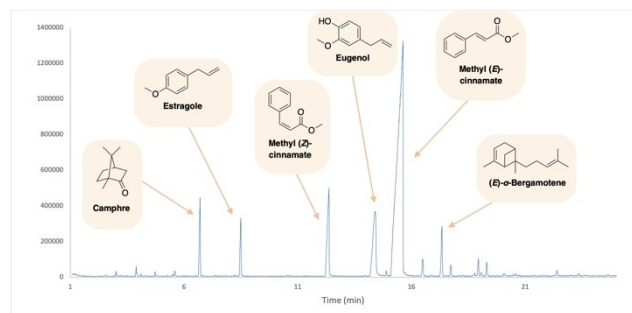


Figure 3. Main components of the distillate of the romba concrete.

tion found in the database of odorous ingredients "The Good Scents Company"^[22] which lists a very large number of ingredients in the field of aromas and perfumes and their olfactory and / or aromatic description.

In addition, GC-O-MS analyses made it possible to focus in more detail on the areas of the chromatogram identified as being of olfactory interest to the perfumer. Several samples of the distillate at different concentrations (1, 5 and 10% in MTBE) were analyzed in order to assess both the largest and smallest peaks, generate their mass spectrum and confirm the assignments listed in Table 4.

The compositions of romba essential oils described in a selection of scientific articles representing the 14 most recent publications have been grouped in Table 5 in order to compare them with the composition of the volatile fraction of the romba extract that we established during this work.^[1-5,10,12,16,23-28] It is worth noting that some essential oils were obtained from leaves, other from leaves and stems, or other from leaves and flowers. This adds to the natural variability. Compounds are classified by increasing retention index (identification by EI-MS spectra comparison and RI determination) and their percentage abundance is reported (quantification by GC-FID).

The essential oils studied in these publications are mostly obtained from a eugenol chemotype (10 out of 14), some of which are composed of more than 70% of this compound. Thymol and linalool chemotypes were also found. The extract we have studied has features of a methyl (*E*)-cinnamate chemotype. Apart from a few compounds present in almost each of the compositions analyzed, such as 1,8-cineole, (*E*)- β -ocimene, eugenol or caryophyllene, the compositions are relatively varying even within the same chemotype.

Principal Component Analysis (PCA) was performed and confirmed the relatively distributed variance, component 1 accounting for only 28.9% and component 2 for 14.3% (Figure 4). Individual contribution of components 1 and 2 were significantly brought by our sample from Madagascar with 62%, followed by a Brazilian sample with 25% and a Vietnamese sample with less than 3%. Compounds contributing strongly to the variance were found to be allo-ocimene, α -terpineol, *trans*-bergamotene, (*Z*)- β -farnesene, β -copaene, δ -2-carene and *p*-cymene, all belonging to the terpenes and terpenoids family.

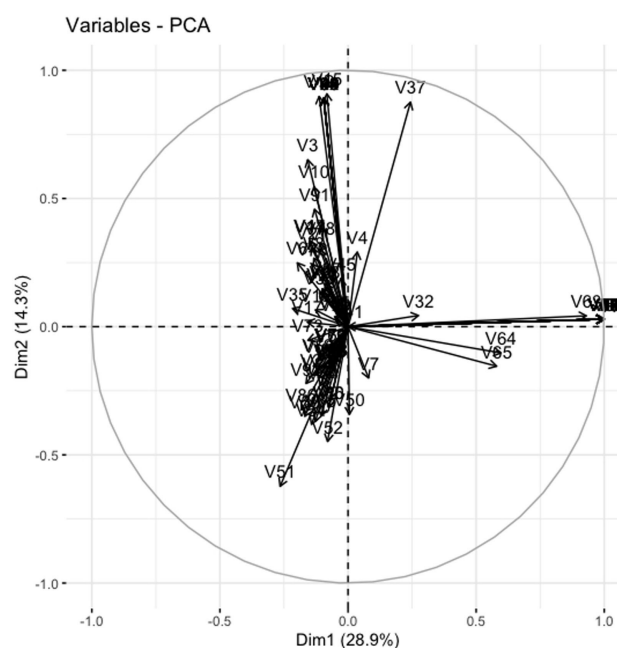


Figure 4. PCA plot of the chemical composition (96 variables) for 14 essential oils.

This diversity of composition can be explained by several factors. Indeed, the composition of an essential oil fluctuates a lot depending on the parts of the plant (leaves, flowers, stem, etc.) used, and can also present a significant natural variability depending on the practices of cultivation and harvesting, the type of soil, the biotope, the climate, the season and the time of the day of harvest.^[29-32] In addition, the type of extraction method can lead to different compositions from the same raw material.

Our sample shows some similarities with essential oils of *Ocimum americanum* which presents also a cinnamate chemotype. This prompted us to examine our data in more details in comparison with the chemical composition of essential oils from aerial parts of plants of the *Ocimum* genus described in the literature.

Possible similarities with *Ocimum americanum* and *Ocimum canum*

Issues of authenticity, conformity and adulteration are not negligible in essential oils and natural extracts.^[33-34] Having in hand a solvent extract and not a botanical specimen, its authenticity as an *Ocimum gratissimum* vs. *Ocimum americanum* or *Ocimum canum* for example was a fair question. A cinnamate chemotype was indeed reported for *O. americanum* from India on the basis of the chemical profile of the essential oil obtained from the whole plant (over 81% for methyl (*E*)- and (*Z*)-cinnamate).^[15] However, beside supposedly from the same chemotype, out of 119 compounds, our sample have only 41 in common with the sample from this study. They differ indeed mostly in the terpene and terpenoids composition.

Table 3. Chemical composition of romba distillate established by MS spectrum and RI comparison (identity) and GC-FID (quantity).

Entry	RI exp.	[RI litt. ^[19–21]	Compound	% (GC-FID)	Chemical class
1	922	921	Tricyclene	0.02	Monoterpene hydrocarbon
2	928	932	α -Pinene	0.03	Monoterpene hydrocarbon
3	941	946	Camphene	0.07	Monoterpene hydrocarbon
4	950	952	Benzaldehyde	tr.	Phenylpropanoid
5	965	969	Sabinene	0.02	Monoterpene hydrocarbon
6	970	974	1-Octen-3-ol	0.2	Aliphatic alcohol
7	978	979	3-Octanone	tr.	Aliphatic ketone
8	984	988	Myrcene	0.08	Monoterpene hydrocarbon
11	999	1002	α -Phellandrene	tr.	Monoterpene hydrocarbon
12	1003	1004	(3Z)-hexenyl acetate	0.03	Aliphatic ester
13	1019	1022	<i>p</i> -Cymene	0.01	Monoterpene hydrocarbon
14	1023	1024	Limonene	0.38	Monoterpene hydrocarbon
15	1024	1026	1,8-Cineole	0.09	Oxygenated monoterpene
16	1028	1026	Benzyl alcohol	tr.	Phenylpropanoid
17	1032	1032	(Z)- β -Ocimene	0.09	Monoterpene hydrocarbon
18	1041	1044	(E)- β -Ocimene	0.03	Monoterpene hydrocarbon
19	1050	1054	γ -Terpinene	0.01	Monoterpene hydrocarbon
20	1077	1083	Fenchone	tr.	Oxygenated monoterpene
21	1079	1085	<i>p</i> -Mentha-2,4-diene	0.03	Monoterpene hydrocarbon
22	1086	1088	Methyl benzoate	tr.	Phenylpropanoid
23	1090	1086	Terpinolene	tr.	Monoterpene hydrocarbon
24	1094	1095	Linalool	0.30	Oxygenated monoterpene
25	1107	1107	Phenyl ethyl alcohol	0.01	Phenylpropanoid
26	1124	1128	<i>allo</i> -Ocimene	tr.	Monoterpene hydrocarbon
27	1134	1141	Camphor	4.04	Oxygenated monoterpene
28	1161	1155	Isoborneol	0.02	Oxygenated monoterpene
29	1192	1195	Estragole	2.73	Phenylpropanoid
30	1242	1247	<i>p</i> -Anisaldehyde	0.05	Phenylpropanoid
31	1247	1254	2-Phenylethyl acetate	0.03	Phenylpropanoid
32	1295	1299	Methyl (Z)-cinnamate	7.37	Phenylpropanoid
33	1338	1348	α -Cubebene	0.03	Sesquiterpene hydrocarbon
34	1350	1356	Eugenol	13.90	Phenylpropanoid
35	1362	1374	α -Copaene	0.32	Sesquiterpene hydrocarbon
36	1378	1376	Methyl (E)-cinnamate	62.39	Phenylpropanoid
37	1398	1393	Vanilline	0.41	Phenylpropanoid
38	1404	1403	Methyleugenol	0.14	Phenylpropanoid
39	1408	1408	(E)-b-Caryophyllene	1.09	Sesquiterpene hydrocarbon
40	1417	1419	β -Cedrene	tr.	Sesquiterpene hydrocarbon
41	1426	1432	α - <i>trans</i> -Bergamotene	2.65	Sesquiterpene hydrocarbon
42	1433	1440	(Z)- β -Farnesene	0.08	Sesquiterpene hydrocarbon
43	1437	1452	α -Humulene	0.59	Sesquiterpene hydrocarbon
44	1451	1454	(E)- β -Farnesene	0.02	Sesquiterpene hydrocarbon
45	1464	1465	<i>cis</i> -Muurola-4(14),5-diene	0.16	Sesquiterpene hydrocarbon
46	1472	1457	Sesquisabinene	0.79	Sesquiterpene hydrocarbon
47	1478	1476	β -Chamigrene	0.65	Sesquiterpene hydrocarbon
48	1487	1483	α -Amorphene	0.01	Sesquiterpene hydrocarbon
49	1490	1493	α -Zingiberene	tr.	Sesquiterpene hydrocarbon
50	1498	1493	<i>trans</i> -Muurola-4(14),5diene	0.04	Sesquiterpene hydrocarbon
51	1509	1511	δ -Amorphene	0.12	Sesquiterpene hydrocarbon
52	1512	1521	β -Sesquiphellandrene	0.11	Sesquiterpene hydrocarbon
53	1532	1532	γ -Cuprenene	0.01	Sesquiterpene hydrocarbon
54	1538	1548	Allyl (E)-cinnamate	0.01	Phenylpropanoid
55	1574	1544	Methyl (Z)- <i>p</i> -methoxycinnamate	0.05	Phenylpropanoid
56	1653	1614	Methyl (E)- <i>p</i> -methoxycinnamate	0.02	Phenylpropanoid
			Monoterpene hydrocarbon	0.77	
			Oxygenated monoterpene	4.45	
			Sesquiterpene hydrocarbon	6.67	
			Aromatic	87.11	
			Others	0.23	
			Total	99.23 %	

O. americanum essential oils from Burkina Faso were described to be rich in 1,8-cineole (45.0–60.2%), camphor and α -terpineol.^[35] With plant from Nigeria, the chemotype was mostly eugenol and farnesol,^[36] while from Rwanda, some linalool-rich essential oils were obtained.^[37] Camphor chemo-

types were described for Indian plant material.^[38] Again in India, chemotypes estragole (methyl chavicol) and citral,^[39] and citral alone (up to 76%) after natural selection, were described.^[40]

The less studied *Ocimum canum* species were described as rich in camphor (60%) and limonene (13%) in essential oils

Table 4. Assignment of olfactory notes to molecules during the GC-O session by comparison with GC/MS results. Red: high interest, orange: moderate interest, green: low interest.

Entry	RI	Olfactory notes	Molecule
1	961	Blackcurrant, passion fruit, fruity	Not detected
2	979	Aldehyde, metallic, beautiful, pure, fresh, pear	1-Octene-3-one
3	1022	Woody, copaiba	<i>p</i> -Cymene
4	1024	Herbal, myrtle, rosemary, sage	<i>L</i> -limonene/1,8-cineole
5	1095	Green, watery, floral, lotus	Linalool
6	1141	Aldehyde, fresh, floral rose	Camphor
7	1195	Anise, fennel	Estragole
9	1247	Anise, almost jasmona	<i>p</i> -Anisaldehyde
10	1254	Blackcurrant buds, very beautiful	2-Phenylethyl acetate
11	1299	Woody, very beautiful, copaiba fat	Methyl (<i>Z</i>)-cinnamate
12	1348	Light grass	α -Cubebene
13	1356	Eugenol	Eugenol
14	1376	White floral fruity, pineapple, jasmine	Methyl (<i>E</i>)-cinnamate
15	1393	Lili, vanillin, vanitrop	Vanilline
16	1452	Woody, spicy, isoeugenol,	α -Humulene
17	1454	Fruity, tropical, passion fruit	(<i>E</i>)- β -Farnesene
18	1521	Woody, blond, cedar of Chile	β -Sesquiphellandrene
19	1548	Slightly spicy	Allyl (<i>E</i>)-cinnamate

from Somalia and India^[41] and in methyl (*Z*)- and (*E*)-cinnamate in *O. canum* from Egypt.^[42] Interestingly, the authors studied enzymatic pretreatment prior to hydrodistillation, an approach that was described with other plant materials to improve distillation yields or improve the quality post-distillation.^[43–45] Although of the same chemotype, the chemical profile was different from that of our Malagasy sample.

The possibility of an error of authentication of our *Ocimum gratissimum* sample was thus ruled out based on these comparisons (a mapping of chemotypes is presented in Figure 5).

Conclusions

As part of this work, the first chemical composition of the volatile fraction of a romba concrete from Madagascar was carried out by GC/MS and GC-FID. In addition, the olfactory evaluation by GC-O was carried out by the internationally renowned master perfumer Christophe Laudamiel, including the first on a sample of Romba of methyl (*E*)-cinnamate chemotype, but also the first on a solvent extract and not an essential oil. This work, supported by GC-O-MS analyses, has thus enabled the identification of important odoriferous compounds in the distillate of this concrete, obtained from leaves and stems of *Ocimum gratissimum* of Madagascar.

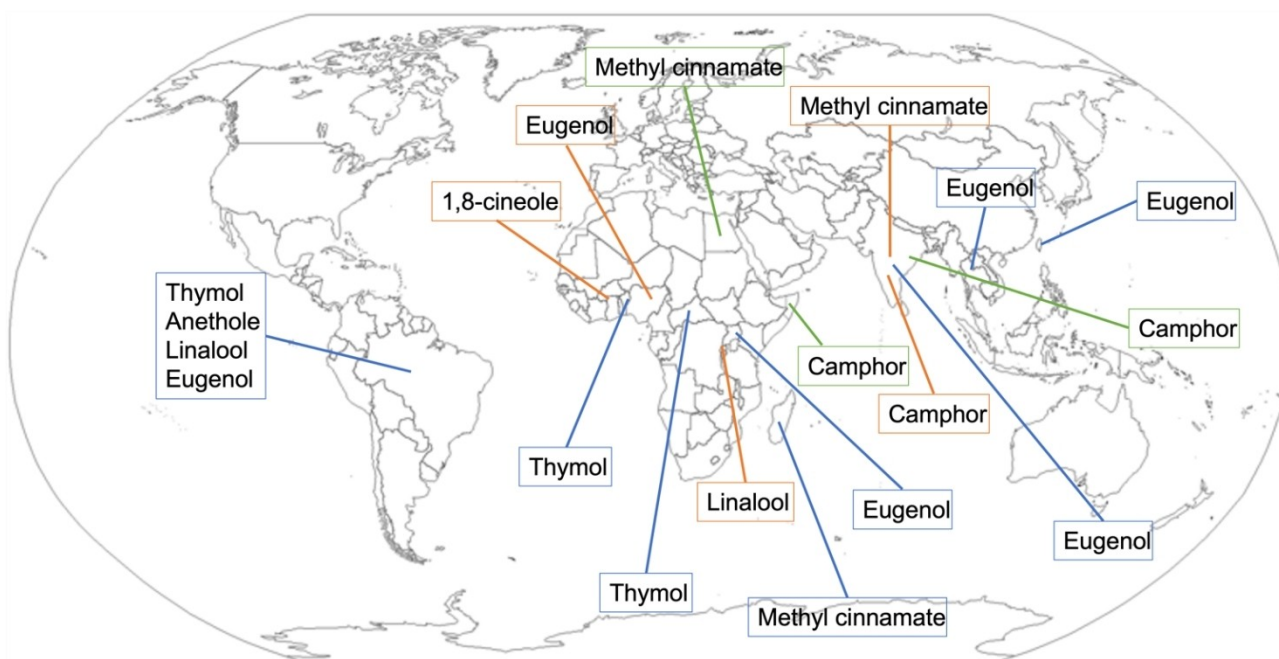


Figure 5. Mapping of chemotypes described from *Ocimum gratissimum* (blue), *Ocimum americanum* (orange), and *Ocimum canum* (green).

Table 5. Comparison of the chemical composition of the romba concrete distillate with literature data.

rt	RI	RI litt.	Compound	Madagascar	Vietnam ²³	Brasil ¹	Unknown ¹⁶	Vietnam ²	Brasil ³	India ⁴	Brasil ¹²	Brasil ²⁴	India ²⁵	Brasil ¹⁰	Taiwan ⁵	Benin ²⁶	Brasil ²⁷	Guangdong ²⁸
				(this study)	wt%													
2.3	922	921	Tricyclene	0.02					2.8			0.47	0.6					
		924	Origanene		0.43													
		939	α -Pinene	0.03		0.08	0.04		0.7	0.2	0.53	3.47	0.1			5.45		
2.57	941	945	Camphene	0.07					0.1	tr.	0.46	0.09				0.12		
2.69	950	952	Benzaldehyde	tr.														
		969	Sabinene	0.02		0.17	0.45		0.4	tr.	0.6	0.21	0.5			0.34		
3.01	970	974	1-Octen-3-ol	0.2									0.5					1.1
		979	β -Pinene			0.43	0.02			0.1	1.25	0.63				4.76		
3.12	978	979	3-Octanone	tr.														
3.21	984	988	β -Myrcene	0.08	0.17	0.14	0.3		1.9	tr.	0.83	0.89				0.18		
		1001	δ -2-Carene													3.89		
3.43	999	1002	α -Phellandrene	tr.					0.4			3.41	0.4					
		1014	α -Terpinene		0.20				2.2				0.3					
3.8	1019	1022	<i>p</i> -Cymene	0.01	0.10				17			2.24	0.8			13.58		
3.9	1023	1024	Limonene	0.38			0.06					1.15	16.63			1		
		1025	β -Phellandrene		0.48													
3.93	1024	1026	1,8-Cineole	0.09		15.16				0.7	21.91	3.36	0.1	9.69	15.4	0.35	3.09	3.7
4.03	1028	1026	Benzyl alcohol	tr.														
4.11	1032	1032	(<i>Z</i>)- β -Ocimene	0.09			0.35					2.62	3.5	1.49		0.304		7.6
4.33	1041	1044	(<i>E</i>)- β -Ocimene	0.03	10.38	0.1	6.79	6.355	0.1	0.1		0.15	0.2					
4.54	1050	1054	γ -Terpinene	0.01	0.36		0.32		30.3	0.2		2.68	0.4			17.52		
4.98	1069	1065	<i>cis</i> -Sabinene hydrate		0.57			0.666	0.9	tr.			2.4					
		1069	4-methylstyrene													1.54		
5.19	1077	1083	Fenchone	tr.								0.86						
5.25	1079	1085	<i>p</i> -Mentha-2,4-diene	0.03														
5.39	1086	1088	Methyl benzoate	tr.														
5.5	1090	1086	Terpinolene	tr.						0.3			0.1					
		1098	<i>trans</i> -Sabinene hydrate										0.6					
5.6	1094	1095	Linalool	0.3		0.34	0.29		0.2		32.95	0.12	0.7	0.42				1
5.91	1107	1107	Phenyl ethyl alcohol	0.01														
6.42	1124	1128	<i>allo</i> -Ocimene	tr.														
6.74	1134	1141	Camphor	4.04						0.3	11.97					0.11		
7.38	1161	1165	Borneol	0.02					0.1	0.1						0.41		
		1166	δ -Terpineol		0.64	0.12						0.06						
		1177	Terpinen-4-ol			0.16	0.75		0.5	0.7	0.79	0.16	1.3			1.75		
		1188	α -Terpineol			0.31	0.06		0.1	tr.	3.36	0.11	0.1			1.93		
8.51	1192	1195	Eugenol	2.73								7.78				0.25		
		1218	<i>endo</i> -Fenchyl acetate									0.1						
		1223	Citronellol									0.17						
		1229	<i>exo</i> -fenchyl acetate									0.17						
		1232	Thymol methyl ether															
		1247	(<i>Z</i>)-Anethole									0.17						
10.36	1242	1247	<i>p</i> -Anisaldehyde	0.05														
		1249	Geraniol									0.27						
10.54	1247	1254	Phenyl ethyl acetate	0.03					0.5									
		1282	(<i>E</i>)-Anethole									34.95						
		1289	Thymol						33.4			4.47				32.88		
		1298	Carvacrol						1.2							1.18		
12.3	1295	1299	Methyl (<i>Z</i>)-cinnamate	7.37														
13.97	1338	1348	α -Cubebene	0.03	0.22								0.2					
14.4	1350	1356	Eugenol	13.9	59.45	74.83	69.08	77.39		57.1	7.42	9.07	52.9	72.26	73.6		70.96	56.4
14.89	1362	1374	α -Copaene	0.32	2.48			1.248		0.6		0.1	2.3		t			2
15.5	1378	1376	Methyl (<i>E</i>)-cinnamate	62.39														
		1387	β -Bourbonene		0.96			0.716		0.5		0.05	0.1					
		1387	β -Cubebene		11.78					0.1								10.9
		1389	β -Elemene		0.54				0.2		0.93	0.12	1.9					
16.26	1398	1393	Vanilline	0.41														
16.51	1404	1403	Methyleugenol	0.14														
16.65	1408	1408	(<i>E</i>)- β -Caryophyllene	1.09	7.00	2.2	3.48	3.16	1.7	14.2	1.68	0.65	1.6	3.8	t	3.55		3.5
17.01	1417	1419	β -cedrene	tr.														
		1421	β -Ylangene															4.99
		1430	β -Copaene				0.32	0.743					0.3					
17.43	1426	1432	<i>trans</i> - α -Bergamotene	2.65							1.07							
17.7	1433	1440	(<i>Z</i>)- β -Farnesene	0.08						tr.			0.1					
17.85	1437	1452	α -Humulene	0.59	0.53	0.32	0.38		0.2	0.1		0.09	0.2					
18.36	1451	1454	(<i>E</i>)- β -Farnesene	0.02														5.5
18.86	1464	1465	<i>cis</i> -Muurolo-4(14),5-diene	0.16														
19.14	1473	1473	Sesquisabinene	0.79														
19.39	1478	1476	β -Chamigrene	0.65														
		1478	γ -Muurolole			0.51	0.11						0.1	0.46				
		1481	γ -Himachalene															2.62

19.68	1487	1483	α -Amorphene	0.01																
		1490	β -Selinene		2.82			1.8	tr.	0.02		4.25		2.61	8.23					
19.80	1490	1493	α -Zingiberene	tr.																
20.12	1498	1493	<i>trans</i> -Muuroala-4(14),5-diene	0.04																
		1496	Ledene																	3.42
		1496	Valencene									1.87								
		1498	α -Selinene		0.85				tr.	0.38	0.1									
		1500	Bicyclodermacrene									2.56								
		1500	α -Muurole		0.25															
		1505	(<i>E,E</i>)- α -Farnesene									1.16								
		1509	α -Bulnesene						15.6											
2053	1509	1511	δ -Amorphene	0.12																
		1513	γ -Cadinene		0.35	0.14			1.05		0.3									1.7
20.64	1512	1521	β -Sesquiphellandrene	0.11																
		1522	δ -Cadinene		1.05		0.44		0.4		0.12	0.7			0.33					
		1522	7- <i>epi</i> - α -Selinene			0.26					0.06									
21.39	1532	1532	γ -Cuprenene	0.01																
21.63	1538	1548	Allyl (<i>E</i>)-cinnamate	0.01																
23.00	1574		Methyl (<i>Z</i>)- <i>p</i> -methoxycinnamate	0.05																
		1578	Spathulenol		0.07			0.3			0.1									
		1582	Caryophyllene oxide		0.27	0.55	1.86	1.101		2.2	0.11				0.37					
		1602	Ledol																	1.2
		1640	α -cadinol								0.07									
		1644	α -Muurolol		0.08						5.18	0.04								
25.82	1653		Methyl (<i>E</i>)- <i>p</i> -methoxycinnamate	0.02																
			TOTAL		99.20	98.28	99.42	84.80	91.82	96.80	93.70	93.13	98.38	73.40	96.80	89.00	94.40	93.31	94.60	

Comparison with literature data on various essential oils of plants from the genus *Ocimum* and from various chemotypes of *Ocimum gratissimum* illustrate the subtle differences between those natural complex mixtures within natural variability.

Experimental Section

Analyses were performed by gas chromatography coupled with mass spectrometry (GC/MS) for identification and by gas chromatography coupled with flame ionization detector (GC-FID) for quantification. The identification of essential oil constituents was carried out by matching the retention indices (RI) determined against a series of alkanes as well as by matching EI-MS mass spectra obtained with databases (NIST20, Wiley6n and internal database). The relative quantification was performed by GC-FID.

The analyses were performed on an Agilent 7820A chromatograph coupled to an Agilent 5977B electron ionization mass selective detector. The method used for each analysis is detailed below:

Column: Teknokroma SAPIENS-5MS capillary column (10 m × 0.1 mm × 0.1 μ m). Sample: essential oil diluted to 1% v/v in ethyl acetate. Injection volume: 0.2 μ L. Split ratio: 1/50. Carrier gas: helium. Injector and source temperatures: 250 °C and 230 °C. MS scan: from 35 to 550 *m/z*. Temperature ramp: 50 °C for 0 min, from 50 to 230 °C with an increase of 10 °C/min then from 230 to 300 °C with an increase of 60 °C/min.

GC-O-FID analysis were performed on a Shimadzu QP2010 chromatograph, coupled with a FID detector and equipped of an olfactory port GL Sciences OP275. Column: Agilent HP-5 50 m × 0.32 mm × 0.52 μ m. Split FID/OP: 1:9 v/v. Injection: 1 μ L. Temperature ramp: 60 °C for 0 min, from 60 to 250 °C with an increase of 5 °C/min, then held at 250 °C for 10 min.

GC-O-MS analysis were performed on a Perkin Elmer Clarus 690 chromatograph, coupled with a Perkin Elmer Clarus SQ8T mass selective detector. Column: Perkin Elmer Elite 5-MS 30m × 0.32 mm × 0.25 μ m. Injection: 1 μ L. Inlet pressure: 23 psi. Split: 72 mL/min. MS–O split: 1:7 v/v. Temperature ramp: 60 °C for 2 min,

from 60 to 200 °C with an increase of 5 °C/min then from 200 to 280 °C with an increase of 8 °C/min, then held at 280 °C for 5 min.

Author Contributions

LG and SA performed the work. SA analyzed the results and wrote the article.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: complex natural substance · chemical analysis · natural variability · olfactometry · chemotypes

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