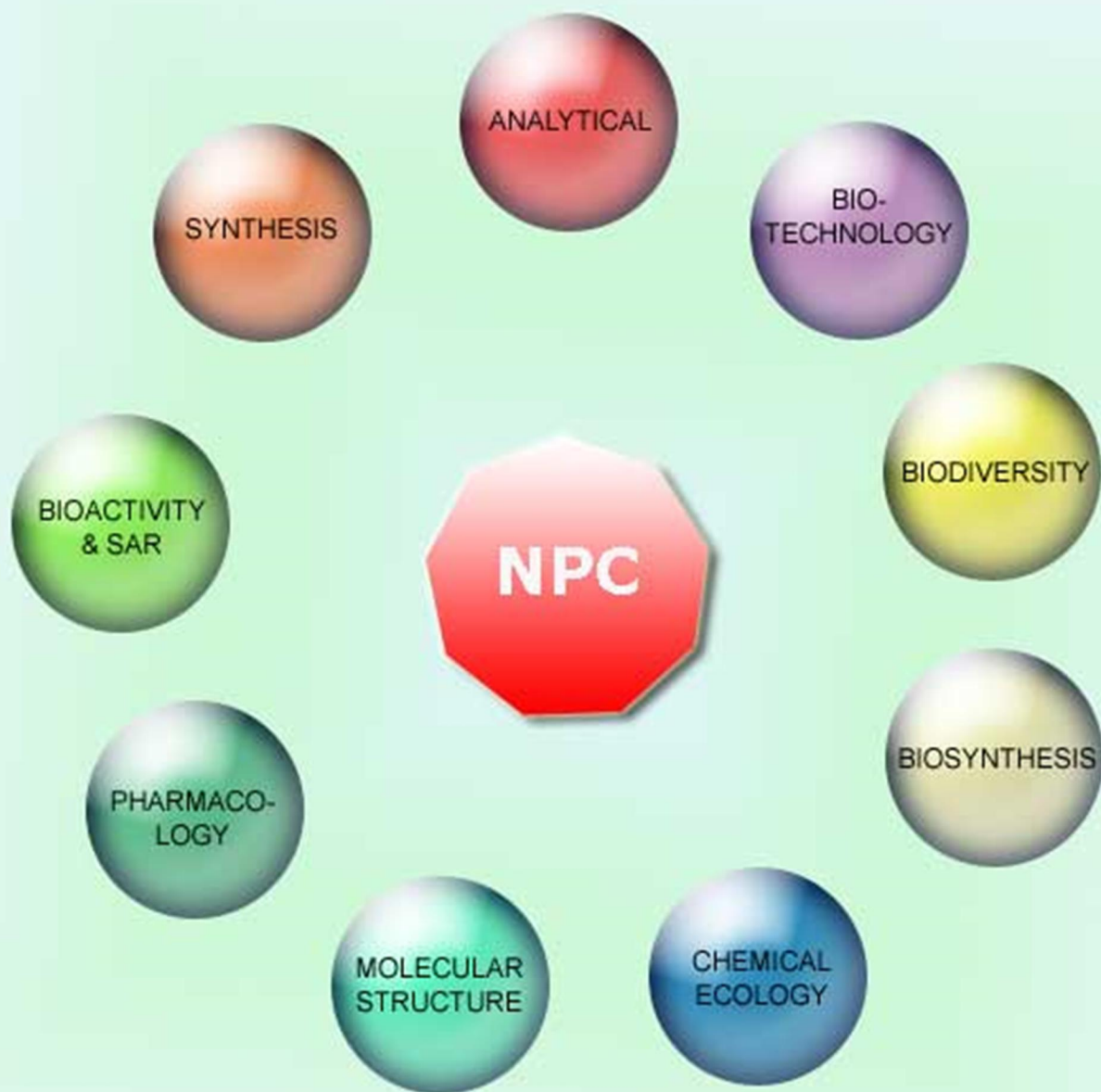


# NATURAL PRODUCT COMMUNICATIONS

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**This Issue is Dedicated to  
Professor Dr Wilhelm Fleischhacker  
On the Occasion of his 85th Birthday**

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## Analysis and Olfactory Description of Four Essential Oils from Vietnam

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The present study evaluates the chemical composition and olfactory description of the essential oils of *Asarum glabrum* Merr., *Calocedrus macrolepis* Kurz, *Cunninghamia lanceolata* (Lamb.) Hook. and *Glyptostrobus pensilis* (Stainton ex D. Don) K. Koch. The essential oils were obtained by hydrodistillation in a Clevenger-type apparatus and analyzed by GC-FID and GC-MS. Concerning their chemical composition, 66, 42, 57 and 21 volatile compounds were identified from dried leaves in the case of *Asarum glabrum* Merr. and wood for the other three, representing 98.7%, 67.2%, 92.0% and 87.5% of the total composition, respectively. The main compounds of *Asarum glabrum* oil were safrole (38.1%), apiole (10.8%) and myristicin (8.0%); of *Calocedrus macrolepis* verbenone (9.3%), piperitone (8.6%),  $\alpha$ -terpineol (6.0%) and (*Z*)- $\beta$ -terpineol (5.3%); of *Cunninghamia lanceolata* oil cedrol (26.3%),  $\alpha$ -terpineol (24.1%) and camphor (7.0%); and of *Glyptostrobus pensilis* oil dihydro-eudesmol isomer (assumed) (18.3%), cedrol (16.4%), occidentalol (13.2%) and elemol (9.0%).

**Keywords:** *Asarum glabrum*, *Calocedrus macrolepis*, *Cunninghamia lanceolata*, *Glyptostrobus pensilis*, Vietnam, GC-FID/MS, Olfactory evaluation.

Vietnam is well known for its wealth of rare endemic flora and therefore many plants are liable to the Red Data Book of Vietnam and a Governmental decree [1]. This is applicable for *Asarum glabrum*, *Glyptostrobus pensilis* and *Calocedrus macrolepis*. Nevertheless, such plants produce essential oils (EO) with interesting compositions. Observing media information, more and more components of EOs are interesting for medicinal treatments or are base material and starters for pharmaceutically important drugs like star anise oil with (*E*)-anethole for the production of +(R)-Tamiflu® [2]. In consciousness of this fact and the knowledge that over 40% of pharmaceutical medications come from plants, the aim of this work was to analyze the EOs of these four species to obtain information on their volatile components [3].

*A. glabrum* Merr. (Aristolochiaceae), common local name in Vietnam is Hoa tiên, is a perennial herb 20 - 30 cm in height with purple when young, later green leaves. Hydrodistillation using a Clevenger-type apparatus resulted in 0.2%, v/w, oil yield. Analytical data by GC-MS are given in Table 1.

The oil is characterized by the presence of the phenylpropanoids safrole, apiole, myristicin and dillapiole; the quantities were 38.1%, 10.8%, 8.0% and 7.8%, respectively. Sesquiterpenes are the next group with around 24%, while monoterpenes are around 8%. The total composition consists of 74.5% phenylpropanoids, 7.9% sesquiterpene ethers, 4.2% sesquiterpenes, 3.5% monoterpene alcohols and 2.6% sesquiterpene alcohols. Two unknown components could not be assigned clearly. It is remarkable that apiole and dillapiole appear in the *Apiaceae* family, but were not found before in the *Aristolochiaceae* family.

*C. macrolepis* Kurz, syn. *Libocedrus macrolepis* (Kurz) Benth. & Hook. (Cupressaceae), is listed as vulnerable (B1 + 2b) in Vietnam.

The common local name is Bách xanh. The tree is straight-boled with a height up to 25 m and a diameter up to 0.8 m. The wood is used for construction, but also for incense and EO distillation [4]. Hydrodistillation using a Clevenger-type apparatus resulted in 0.3%, v/w, oil yield. Analytical data by GC-MS are given in Table 2.

This oil is dominated by monoterpene alcohols (25.8%) and monoterpene ketones (26.7%), with a further 7.1% of monoterpene ethers and 2.3% of monoterpene esters. The main component was the monoterpene ketone verbenone (9.3%), followed by piperitone (8.6%),  $\alpha$ -terpineol (6.0%) and *cis*-beta-terpineol (5.3%). There is only one sesquiterpene hydrocarbon, cadalene (0.4%). The terpeneol family, with 13.5%, is responsible for the odor, together with carvacrol and thymol methylether.

*C. lanceolata* (Lamb.) Hook. (Cupressaceae) is a tree with pyramidal habitus with a height up to 50 m. The common local name in Vietnam is Sa mu đầu. The wood is used for house construction and production of coffins. This is because the wood is resistant to termites and rot [5]. Hydrodistillation using a Clevenger-type apparatus resulted in 0.2%, v/w, oil yield. Analytical data by GC-MS are given in Table 3.

The oil showed highest values for cedrol (26.3%),  $\alpha$ -terpineol (24.1%), camphor (7.0%), borneol (4.3%) and *trans*-dihydro- $\alpha$ -terpineol (4.3%). In total, monoterpene alcohols (42.4%), sesquiterpene alcohols (32.5%), monoterpene ketones (9.3%) and sesquiterpene hydrocarbons (3.8%) were detected.

The found values are not in accordance with formerly published papers, especially for cedrol. Shie and Sumimoto [6] reported a value of 60.5% for cedrol in an EO that was hydrodistilled and then separated by a chromatographic method into 5 fractions. Su *et al.*

**Table 1:** Composition (in %) of the EO from dried leaves of *Asarum glabrum* from Vietnam by GC-FID and GC-MS

N°	Compound	RI <sup>#</sup>	%
1	$\alpha$ -Pinene	943	0.01
2	Camphene	959	0.01
3	$\beta$ -Pinene	988	0.06
4	Myrcene	993	0.01
5	$\alpha$ -Phellandrene	1011	0.01
6	<i>p</i> -Cymene	1032	0.01
7	Limonene	1037	0.08
8	1,8-Cineole	1041	0.2
9	( <i>E</i> )-Ocimene	1050	0.01
10	$\gamma$ -Terpinene	1066	0.01
11	<i>cis</i> -Linalool oxide	1079	0.02
12	<i>trans</i> -Linalool oxide	1094	0.01
13	<i>p</i> -Cymenene	1097	0.02
14	Linalool	1102	1.3
15	$\alpha$ -Fenchol	1126	0.04
16	1,1-Diisobutoxypentane	1157	0.1
17	Camphor	1160	0.03
18	Borneol	1180	0.3
19	Terpinen-4-ol	1190	0.4
20	<i>p</i> -Cymen-8-ol	1194	0.06
21	$\alpha$ -Terpineol	1201	1.4
22	$\gamma$ -Terpineol	1208	0.03
23	Myrtenal	1210	0.01
24	Citronellol	1230	0.02
25	Nerol	1234	0.02
26	Thymol methyl ether	1240	0.01
27	Isobornyl formate	1243	0.05
28	Linalyl acetate	1257	0.06
29	( <i>E</i> )-Anethole	1264	0.03
30	Bornyl acetate	1298	0.09
31	Safrol	1302	38.1
32	$\delta$ -Elemene	1354	0.04
33	Terpinyl acetate	1359	3.6
34	Eugenol	1368	0.3
35	Unknown 1	1378	0.6
36	Unknown 2	1391	1.0
37	$\alpha$ -Copaene	1397	0.07
38	Methyl eugenol + $\beta$ -Elemene	1406	2.1
39	$\alpha$ -Santalene	1438	0.05
40	$\alpha$ -Cedrene	1443	0.06
41	( <i>E</i> )- $\beta$ -Caryophyllene	1447	0.05
42	<i>trans</i> - $\alpha$ -Bergamotene	1451	0.5
43	<i>trans</i> - $\beta$ -Farnesene	1461	0.3
44	Aromadendren	1467	0.08
45	Myristicin	1482	0.04
46	ar-Curcumene	1495	1.5
47	( <i>E</i> )-Methyl isoeugenol	1502	0.2
48	Sarisane	1509	3.6
49	$\beta$ -Selinene	1513	0.5
50	$\alpha$ -Selinene + $\beta$ -Curcumene	1522	0.9
51	Sesquicineole	1531	7.8
52	Myristicin	1535	8
53	$\delta$ -Cadinene	1543	0.2
54	Elemicin	1558	0.8
55	( <i>E</i> )-Nerolidol	1571	0.4
56	( <i>Z</i> )-Isoelemicin	1577	1.7
57	2,3,4,5 Tetramethoxyallylbenzene	1602	0.08
58	Spathulenol	1607	0.1
59	( <i>Z</i> )-Asarone	1622	0.01
60	Dillapiol	1640	7.8
61	Alismol	1654	0.2
62	$\alpha$ -Acorenol	1661	0.1
63	$\beta$ -Acorenol	1678	0.02
64	$\alpha$ -Bisabolol oxide B	1681	0.07
65	( <i>E</i> )-Asarone	1685	1.1
66	5- <i>epi</i> - $\beta$ -Bisabolol	1692	0.2
67	Apiole	1696	10.8
68	$\alpha$ -Bisabolol	1701	1.5
	total		98.7

**Table 2:** Composition (in %) of the EO from the wood of *Calocedrus macrolepis* from Vietnam by GC-FID and GC-MS.

N°	Compound	RI <sup>#</sup>	%
1	1-Methyl-cyclohexa-1,3-diene	771	0.03
2	$\alpha$ -Pinene	943	0.02
3	$\alpha$ -Fenchene	948	0.1
4	Camphene	957	0.05
5	$\alpha$ -Methylstyrene	988	0.2
6	2,3-Dehydro-1,8-cineole	998	0.1
7	2,6-Dimethyl-6-hepten-2-ol	1011	1.0
8	1,4-Cineole	1021	0.5
9	<i>p</i> -Cymene	1031	0.5
10	Limonene	1037	0.1
11	1,8-Cineole	1041	0.7
12	<i>m</i> -Cymenene	1089	0.07
13	2-Phenyl-2-propanol	1092	2.1
14	<i>p</i> -Cymenene	1097	0.2
15	<i>trans</i> -Sabinene hydrate	1111	0.3
16	$\alpha$ -Fenchocamphorone	1118	1.6
17	<i>trans</i> - <i>p</i> -Menth-2-en-1-ol	1127	1.5
18	$\alpha$ -Fenchol	1131	1.5
19	Terpineol-1	1143	1.8
20	<i>cis</i> -beta-Terpineol	1154	5.3
21	Camphor	1159	3.7
22	Pinocamphone + <i>trans</i> -beta-Terpineol	1174	4.8
23	$\delta$ -Terpineol	1178	0.4
24	<i>p</i> -Methylacetophenone + (iso)Pinocampheol	1182	1.4
25	<i>p</i> -Cymen-8-ol	1187	0.2
26	<i>p</i> -Cymen-9-ol	1189	1.0
27	$\alpha$ -Terpineol	1201	6.0
28	2- $\alpha$ -Hydroxy-1,8-cineole	1219	0.8
29	Verbenone	1224	9.3
30	3- $\alpha$ -Hydroxy-1,8-cineole	1234	3.1
31	Thymol methyl ether	1250	2.8
32	<i>cis</i> -Myrtenol	1258	0.1
33	Piperitone	1268	8.6
34	Phellandral	1291	0.3
35	Thymol	1296	0.2
36	Methyl myrtenate	1302	0.7
37	Carvacrol	1307	1.8
38	Carvone	1313	0.4
39	Methyl thujate	1335	1.6
40	1,3-Dimethoxy-5-(1-methylethyl)-benzene	1376	1.2
41	Carvone hydrate	1440	0.8
42	Cadalene	1702	0.4
	total		67.2

published a value for cedrol of 58.3%, but for *C. lanceolata* var. *konishii* [7]. Wang *et al.* only found 4.9% of cedrol [8].

*G. pensilis* (Stainton ex D. Don) K. Koch (Cupressaceae) is listed in group I of the "Rare and Precious Flora and Fauna" in Vietnam. The common local name in Vietnam is Thù yàng. The tree possesses a pyramidal crown; the smallest branches are usually deciduous, reaching a height of 20 m. The wood was previously used for construction and craftwork and is described as water resistant [9]. Hydrodistillation using a Clevenger-type apparatus resulted in 0.3%, v/w, oil yield. Analytical data by GC-MS are given in Table 4.

This oil is dominated by a high amount of sesquiterpenoids and lacks monoterpenes. Sesquiterpene alcohols (76.8%), sesquiterpene hydrocarbons (9.7%) and sesquiterpene epoxides (0.9%) were detected. The highest values were for dihydro-eudesmol isomer (18.3%), cedrol (16.4%), occidentalol (13.2%), elemol (8.9%) and  $\alpha$ -cedrene (6.1%). All these compounds are responsible for this woody and fine odor.

## Experimental

**Plant material:** Leaves of *A. glabrum* were collected in Hương Sơn District, Hà Tĩnh Province; wood of *C. macrolepis* in Pu Mát

**Table 3:** Composition (in %) of the EO from the wood of *Cunninghamia lanceolata* from Vietnam by GC-FID and GC-MS.

N°	Compound	RI <sup>#</sup>	%
1	Tricyclene	930	tr
2	$\alpha$ -Pinene	941	0.3
3	$\alpha$ -Fenchene	955	0.04
4	Camphene	958	0.2
5	Sabinene	980	0.01
6	2,3-Dehydro-1,8-cineole	996	0.04
7	1,4-Cineole	1020	0.03
8	<i>p</i> -Cymene	1030	0.05
9	Limonene	1035	0.1
10	1,8-Cineole	1039	0.1
11	Fenchone	1097	2.0
12	$\alpha$ -Fenchol	1124	2.2
13	<i>cis</i> -Linalool oxide pyranoid	1136	0.1
14	Terpineol-1	1141	0.08
15	<i>trans</i> -Dihydro- $\alpha$ -terpineol	1153	4.3
16	Camphor	1158	7.0
17	Camphene hydrate	1163	0.3
18	Isoborneol	1171	1.0
19	<i>p</i> -Mentha-1,5-dien-8-ol	1175	0.1
20	Borneol	1179	5.2
21	Terpinen-4-ol	1188	0.4
22	<i>p</i> -Cymen-8-ol	1192	1.1
23	$\alpha$ -Terpineol	1201	24.1
24	2- $\alpha$ -Hydroxy-1,8-cineol	1218	0.4
25	Verbenone	1222	0.04
26	Citronellol	1225	0.02
27	Fenchyl acetate	1228	0.05
28	3- $\alpha$ -Hydroxy-1,8-cineol	1233	0.9
29	Bornyl formate	1242	0.03
30	Piperitone	1266	0.3
31	<i>cis</i> -Myrtenol	1274	0.07
32	Methyl myrtenate	1306	0.5
33	6-Vinyl-2,2,6-trimethyl-2H-tetrahydropyran-3-ol	1314	1.0
34	Terpinyl acetate	1357	0.1
35	<i>trans</i> - <i>p</i> -Menth-6-en-2,8-diol	1390	1.
36	<i>trans</i> - <i>p</i> -Menth-6-en-2,8-diol isomer	1393	0.6
37	Carvone hydrate	1439	1.1
38	$\alpha$ -Cedrene	1441	2.7
39	$\alpha$ -Cedrene	1451	0.7
40	Thujopsene	1459	0.1
41	ar-Curcumene	1494	0.07
42	Cuparene	1532	0.2
43	Elemol	1569	0.1
44	Longicamphenilone	1599	0.08
45	$\alpha$ -Cedrene epoxide	1611	0.07
46	Caryophyllene oxide	1615	0.1
47	Widdrol	1635	2.8
48	Cedrol	1640	26.3
49	<i>epi</i> -Cedrol	1654	0.8
50	$\gamma$ -Eudesmol	1659	0.3
51	$\tau$ -Muurool + $\tau$ -cadinol	1667	0.5
52	$\delta$ -Cadinol	1670	0.3
53	$\alpha$ -Cadinol	1680	1.0
54	Acorenone	1691	0.2
55	$\alpha$ -Bisabolol	1700	0.5
56	Junicedranone	1712	0.1
57	Cedryl acetate	1801	0.4
	total		92.0

Tr = trace (&lt; 0.01)

National Park, Nghe An Province; wood of *C. lanceolata* from Pu Hoat Nature reservation, Nghe An Province; and wood of *G. pensilis* from DarLac Province. Collection was in May 2013. Botanical identification was performed by Dr Do N. Dai. Voucher specimens DND 912, DND 914, DND 915 and DND 916, respectively were deposited at the Botany Museum, Vinh University, Vietnam.

**EO distillation and analysis:** Leaves of *A. glabrum* were dried at room temperature (22°C). Wood samples of *C. macrolepis*, *C.*

**Table 4:** Composition (in %) of the EO from the wood of *Glyptostrobus pensilis* from Vietnam by GC-FID and GC-MS.

N°	Compound	RI <sup>#</sup>	%
1	$\alpha$ -Duprezianane	1411	0.2
2	Sibirene	1425	0.1
3	$\alpha$ -Cedrene	1442	6.1
4	$\beta$ -Cedrene	1452	1.7
5	4,5- $\alpha$ -Eudesmane	1512	0.8
6	Cuparene	1533	0.8
7	Dihydro-eudesmol isomer (assumed)	1540	18.3
8	Elemol	1569	8.9
9	Occidentalol	1571	13.2
10	Caryophyllene alcohol	1602	0.7
11	$\alpha$ -Cedrene epoxide	1611	0.4
12	Caryophyllene oxide	1616	0.5
13	Widdrol	1635	4.0
14	Cedrol	1640	16.4
15	6- <i>epi</i> -Cubenol	1655	2.5
16	$\gamma$ -Eudesmol	1660	1.5
17	$\beta$ -Eudesmol	1682	2.9
18	Dihydro-eudesmol	1692	5.7
19	Khushiol	1721	1.0
20	Occidol	1864	0.6
21	Manool	2093	1.1
	total		87.5

**Table 5:** Odor descriptions of the EOs of *A. glabrum*, *C. macrolepis*, *C. lanceolata* and *G. pensilis*.

<i>Asarum glabrum</i> leaves	herbal, aromatic, somewhat spicy, celery connotation, later balsamic slightly woody.
<i>Calocedrus macrolepis</i> wood	Fresh, cedar like, warm woody, herbal touch, later soft balsamic with little woody-smoky connotation.
<i>Cunninghamia lanceolata</i> wood	Soft woody, slightly terpeny top with fresh and green connotation, later soft woody, fine cedar note.
<i>Glyptostrobus pensilis</i> wood	Tender warm woody notes reminding of cedar and cypress, later balsamic with slight burning note

*lanceolata* and *G. pensilis* were crushed and ground. The EOs were hydrodistilled for 3 h at normal pressure according to the Vietnamese Pharmacopoeia [10]. The obtained oil was stored under refrigeration until sent for analysis.

GC-FID and GC-MS analyses were simultaneously performed on a Thermo Fisher Scientific Trace GC Ultra using a MS-FID splitter consisting of a quartz Y-splitter and a 20 cm  $\times$  0.1 mm ID fused silica restrictor column as an inlet to the GC-MS interface and a 1 m  $\times$  0.25 mm deactivated fused silica column serving as a transfer line to the FID detector. The split/splitless injector was heated at 230°C and connected to a 50 m  $\times$  0.25 mm  $\times$  1.0  $\mu$ m SE-52 capillary column (made and tested for deactivation and separation efficiency in our lab). The FID detector operated at 250°C. EO (0.1  $\mu$ L) was injected with a 0.5  $\mu$ L plunger-in-needle syringe at a split ratio of 1:100 using a TriPlus RSH Autosampler. For substance identification, a Thermo Fisher Scientific ISQ Mass Spectrometer was used with GC-MS interface heating at 250°C, ion source 230°C, EI mode at 70 eV, filament 50  $\mu$ A, scan range 40–500 amu. The following temperature program was used: 60°C for 1 min, heating to 230°C at a rate of 3°C/min, and 230°C for 12.3 min. The carrier gas was helium 5.0 with a constant flow rate of 1.5 mL/min.

Thermo Xcalibur 2.2 software was used for identifying the compounds by correlating mass spectra to databases of NIST 08 (National Institute of Standards and Technology, Gaithersburg, Maryland), Wiley Registry of Mass Spectral Data 8th Edition (Wiley, Hoboken, New Jersey), Adams [11], MassFinder terpenoids library (Hochmuth, Hamburg, Germany) and our own library. Retention indices determined according to [12,13]. Quantification was performed using normalized peak area calculations of the FID chromatogram without – by first approximation – relative FID response factors. All analyses were made in triplicate and the media value was used.

**Olfactory evaluation:** For olfactory evaluation, one droplet of each EO sample was applied onto commercially available paper blotters.

Each sample was examined by a trained professional perfumer and two aroma-chemists over 90 min to control odor progression.

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