

## Evaluation of Volatile Constituents of *Cochlospermum angolense*

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The chemical composition of the essential oils obtained from the leaves and roots of *Cochlospermum angolense* (Welw) growing wild in Angola was analyzed for the first time by capillary gas chromatography (GC) and gas chromatography/mass spectrometry (GC-MS). The investigation led to the identification of 67 and 130 compounds from the leaves and roots, respectively. Both oils were strongly characterized by the presence of sesquiterpenoids (68.8% in the leaves and 53.2% in the roots), while monoterpenoids were present in minor percentages (9.8% in the leaves and 26.2% in the root). The main constituents of the leaves were germacrene D (9.4%),  $\alpha$ -cadinol (7.4%) and 10-*epi*-cubenol (6.2%), while the most abundant compounds in the root essential oil were the sesquiterpenes  $\beta$ -caryophyllene (19.7%) and isoborneol (6.6%). The analysis by HS-SPME of the roots, leaves, fruits and seeds were also reported for the first time. Different volatile profiles were detected.

**Keywords:** *Cochlospermum angolense*, Cochlospermaceae, volatile constituents, HS-SPME, GC-MS, essential oil,  $\beta$ -caryophyllene, germacrene D,  $\alpha$ -cadinol.

Cochlospermaceae, a small family related to the Bixaceae, comprises 17 species grouped into 2 genera: *Cochlospermum* and *Amoreuxia* [1a]. The Cochlospermaceae was studied for the first time by Planchon (1847), whose system is followed today for the botanical systematic approach to these plants. The main diagnostic difference between the two genera is the symmetry of the flower, that is actinomorphic in *Cochlospermum* and zygomorphic in *Amoreuxia*. *Cochlospermum* includes 13 species worldwide distributed mainly across the tropical areas (6 species grow in Africa, 4 in America, 2 in Australia and 1 in Asia). *C. angolense*, *C. planchonii*, *C. regium*, *C. religiosum*, *C. tinctorium* and *C. vitifolium* are reported in the literature for their biological activities and their uses in traditional medicine [1b]. Among these, the three endemic African species, *C. angolense*, *C. planchonii* and *C. tinctorium*, are interesting as hepatoprotective and for their use in treatment of uncomplicated malaria. The antiplasmodial activity of these extracts was confirmed by *in vitro* and *in vivo* tests [1c,2a,2b]. Furthermore, the essential oils obtained by hydrodistillation of roots and leaves of *C. planchonii* and *C. tinctorium* showed the same activity *in vitro* [3,4]. The increasing number of infections and plasmodial cross-resistance encouraged research into new natural bioactive compounds against malaria parasites.

*C. angolense* Welw. ex Oliv. is a wild tree growing in Angola and southern Guinea. *Burututu* is a decoction of the roots of *C. angolense* used in Angolan folk medicine. Its bark is also utilized to make coarse cordage [1b]. The phytochemical profile of the volatile compounds is not recorded in the literature. For this reason this work was focused on the characterization of the volatile constituents from different parts of this tree.

The essential oils (EOs) obtained from the leaves and roots and the SPME profiles of the leaves, roots, fruits and seeds of *C. angolense* collected in Angola were analyzed by GC-MS in order to evaluate the chemical composition of the various volatile fractions. The relative concentrations of the identified components are shown in Table 1 according to their linear retention index (LRI) on a HP-5 column. The yields of EOs obtained by hydrodistillation were 0.01% v/w (dry weight) for both plant materials. GC and GC/MS analysis of the leaf and root essential oils led to the identification of 67 and 130 compounds, respectively, representing around 92 and

96% of the total oils. Sesquiterpene hydrocarbons ( $37.5 \pm 0.85\%$ ) and oxygenated sesquiterpenes ( $31.2 \pm 0.59\%$ ) were the most abundant chemical classes observed in the oil from leaves, while monoterpene hydrocarbons ( $7.5 \pm 0.21\%$ ), oxygenated monoterpenes ( $2.3 \pm 0.05\%$ ) and aliphatic compounds ( $12.8 \pm 0.25\%$ ) were present in lesser percentages. Germacrene D ( $9.4 \pm 0.26\%$ ),  $\alpha$ -cadinol ( $7.4 \pm 0.23\%$ ), 10-*epi*-cubenol ( $6.2 \pm 0.07\%$ ) and *n*-nonane ( $6.3 \pm 0.26\%$ ) were the major volatile constituents.

The root EO contained the same chemical classes observed for the leaf EO (hydrocarbons  $31.9 \pm 0.77\%$  and oxygenated sesquiterpenes  $21.2 \pm 0.65\%$ ), together with a relatively high amount of hydrocarbon and oxygenated monoterpenes ( $4.6 \pm 0.22\%$  and  $21.6 \pm 0.60\%$ , respectively). The aliphatic hydrocarbons were  $13.8 \pm 0.48\%$ .  $\beta$ -Caryophyllene ( $19.7 \pm 0.73\%$ ), isoborneol ( $6.6 \pm 0.29\%$ ),  $\alpha$ -humulene ( $5.6 \pm 0.11\%$ ), caryophyllene oxide ( $5.1 \pm 0.34\%$ ) and 1,8-cineole ( $5.3 \pm 0.22\%$ ) were the main constituents identified in the root EO. The volatile profiles obtained from the HS-SPME of the roots, leaves, fruits and seeds are shown in Table 1. Fifty-five compounds (92.8 %) were identified in the leaf oil, where sesquiterpene hydrocarbons represented the main class of constituents (49.0%). Germacrene D (22.8%),  $\beta$ -pinene (19.7%),  $\alpha$ -pinene (11.9%), limonene (6.2%) and  $\delta$ -elemene (6.1%) were the constituents detected at higher percentages. The main fraction of the root volatiles was represented by sesquiterpene hydrocarbons (93.3%), with high levels of  $\beta$ -caryophyllene (57.3%), cyperene (16.1%) and  $\gamma$ -humulene (9.7%) that characterized this aromatic profile. The analysis of HS of fruits and seeds showed monoterpene hydrocarbons as the most abundant chemical class (93.3% in the fruits and 93.8% in the seeds). High percentages of  $\alpha$ -pinene (42.5%) and  $\beta$ -pinene (40.9%) characterized the HS of the fruits, while  $\beta$ -pinene (30.8%) and myrcene (35.6%) prevailed in the aroma of the seeds.

The volatile composition of the EOs from the leaves and roots of *C. angolense* showed qualitative and quantitative differences. Sesquiterpenes were the main constituents quantitatively, and were quite similar for both oils (68.7% in the leaves and 53.2% in the roots, see Table 2). Conversely the percentages of oxygenated and non-oxygenated derivatives of either monoterpenes

**Table 1:** GC-MS analysis of the leaf and root EOs and the HS GC-MS analysis of the leaves, roots, fruits and seeds of *Cochlospermum. Angolense*.

Compound	Essential Oils				Headspace			
	L.R.I. <sup>a</sup>	L.R.I. <sup>b</sup>	Leaves	Roots	Leaves	Roots	Fruits	Seeds
2-(E)-Hexenal	847	1220	0.2 ± 0.01	0.6 ± 0.04				
2-Methoxy pyrazine	895			tr				
n-Nonane	900		6.3 ± 0.26				tr	
Heptanal	901			0.1 ± 0.01				
Santolina triene	906			tr				
(2E,4E)-Hexadienal	910			tr				
Isocitronellene	924			0.3 ± 0.02				
α-Thujene	930	1035			0.2	tr		
4,5-Dimethyl thiazole	935			tr		tr		
α-Pinene	939	1029	1.2 ± 0.14		11.9	0.9	42.5	12.3
Camphene	954	1012	0.1 ± 0.03	0.7 ± 0.06	tr	0.7	0.6	
Benzaldehyde	960			0.3 ± 0.03				
Thuja-2,4(10)-diene	960		0.4 ± 0.02					
n-Heptanol	967			0.6 ± 0.04				
Unknown	971		0.2 ± 0.01					
Pentyl propanoate	972			2.0 ± 0.20				
β-Pinene	979	1112	3.7 ± 0.26	0.7 ± 0.07	19.7	0.7	40.9	30.8
3-Octanone	984	1380	tr					
6-Methyl-5-hepten-2-one	986	1336	tr	0.2 ± 0.02		0.2		
Myrcene	991	1170	0.3 ± 0.01	tr	tr		1.70	35.6
Mesitylene	996		0.3 ± 0.02		5.0			
n-Decane	1000		0.5 ± 0.01					
α-Phellandrene	1003	1150	tr		0.2			
α-Terpinene	1017	1189	tr		tr			
p-Cymene	1026	1287	tr	0.5 ± 0.03	tr	0.1	3.1	3.0
Limonene	1029	1198	1.5 ± 0.01	0.6 ± 0.04	6.2	0.3	4.2	
β-Phellandrene	1030	1218						11.8
1,8-Cineole	1031	1186	tr	5.3 ± 0.52	tr	0.4		
Benzene acetaldehyde	1042	1625		0.4 ± 0.06				
γ-Terpinene	1055	1256	0.2 ± 0.00	0.2 ± 0.01	0.2	tr	tr	0.3
m-Tolualdehyde	1068			0.3 ± 0.03				
cis-Sabinene hydrate furanoid	1070		tr		tr			
trans-Linalool oxide furanoid	1072			0.4 ± 0.03		tr		
cis-Linalool oxide	1084			1.1 ± 0.03		tr		
Terpinolene	1089	1284	0.1 ± 0.00		tr			
Linalool	1097	1547	0.6 ± 0.02	0.7 ± 0.05	tr	tr		
Perillene	1101							0.5
n-Nonanal	1101	1385	0.1 ± 0.00	0.7 ± 0.07	tr	0.12		0.6
cis-Rose oxide	1108			tr				
endo-Fenchol	1118			0.1 ± 0.00				
2-Ethyl hexanoic acid	1122			tr				
α-Campholenal	1126			tr			0.3	
cis-p-Menth-2,8-dien-1-ol	1138	1638		tr				
trans-Pinocarveol	1139			0.1 ± 0.01		tr	0.3	
Camphor	1144			2.3 ± 0.21		0.2		
4-Keto-isphorone	1145							1.0
Veratrole	1146			tr				
(Z)-Tagetone	1152			tr				
Nerol oxide	1158			0.2 ± 0.02				
2-Acetyl-3-ethyl-pyrazine	1160			tr				
Isoborneol	1162	1715		6.6 ± 0.49		tr		
Pinocarvone	1165						0.4	
Borneol	1169	1718	0.2 ± 0.02		0.3			
2,4-Dimethyl benzaldehyde	1175			0.9 ± 0.07		tr		
4-Terpineol	1177	1611	0.8 ± 0.06	0.7 ± 0.06	tr	tr	0.1	
p-Methyl acetophenone	1183	1607		0.1 ± 0.01				
α-Terpineol	1189		0.8 ± 0.05	1.9 ± 0.02	tr	tr	0.2	
Myrtenol	1196	1596		0.1 ± 0.00			0.2	
n-Decanal	1201	1484		0.2 ± 0.02		0.2	0.2	1.0
Verbenone	1205	1715		0.5 ± 0.03				
neoiso-Dihydro carveol	1229			0.1 ± 0.01				
Nerol	1230			0.2 ± 0.02		tr		
Isobornyl formate	1235			0.1 ± 0.01				
Unknown	1241			0.3 ± 0.01				
Linalyl acetate	1257	1665		0.3 ± 0.01		0.2		
Unknown	1280			0.3 ± 0.02				
Isobornyl acetate	1286			tr		tr		
Bornyl acetate	1289		tr		tr			
1-Tridecene	1292			0.1 ± 0.00		tr		
n-Undecanal	1307		tr					
δ-Elemene	1338		1.2 ± 0.06		6.1	0.2	tr	
α-Cubebene	1351	1466	0.2 ± 0.02		0.5	0.4		
Neryl acetate	1362			0.7 ± 0.02		0.2		
α-Ylangene	1375	1493	0.2 ± 0.01		0.7		0.1	
α-Copaene	1377	1521	0.2 ± 0.01	0.5 ± 0.15	0.5	1.9	0.7	
β-Bourbonene	1388	1535	1.1 ± 0.04		4.6		0.6	
β-Elemene	1391	1660	1.8 ± 0.03		5.3		0.1	
Unknown	1395					0.8		
Cyperene	1399			1.5 ± 0.07		16.1		
(Z)-Isoeugenol	1407			0.4 ± 0.02		tr		
Dodecanal	1409		tr		tr			0.7
α-Gurjunene	1410	1602	tr		tr	tr		
β-Caryophyllene	1419	1604	1.3 ± 0.02	19.7 ± 0.73	3.4	57.3		0.3
β-Ylangene	1421						0.4	
β-Copaene	1432			tr		0.6	0.4	
β-Gurjunene	1434	1612	0.7 ± 0.04		tr	t		
trans-α-Bergamotene	1435			tr				

$\gamma$ -Elemene	1437	1650		t		0.3			
$\alpha$ -Humulene	1455	1642		5.6 $\pm$ 0.11		9.7			
<i>cis</i> -Muurolo-4(14),5-diene	1467			0.1 $\pm$ 0.00					
$\gamma$ -Gurjunene	1477			0.1 $\pm$ 0.01		0.2			
$\gamma$ -Muurolole	1480	1704	1.4 $\pm$ 0.04	tr	0.1	0.9	tr		
Germacrene D	1485	1722	9.4 $\pm$ 0.25	0.3 $\pm$ 0.03	22.8	1.2	0.1	0.3	
$\beta$ -Selinene	1490	1715	5.8 $\pm$ 0.04	1.4 $\pm$ 0.21	0.1	0.3	tr		
2-Tridecanone	1496			3.6 $\pm$ 0.34		0.9			
$\gamma$ -Amorphene	1496		0.4 $\pm$ 0.01		0.2				
$\alpha$ -Selinene	1498	1698	1.9 $\pm$ 0.04	tr	0.4	0.5			
<i>n</i> -Pentadecane	1500		2.5 $\pm$ 0.03		tr				
$\alpha$ -Muurolole	1500	1740	0.6 $\pm$ 0.01	tr	0.7	0.4	0.1		
<i>trans</i> - $\beta$ -Guaiene	1503	1685	2.6 $\pm$ 0.05		0.1				
$\gamma$ -Patchoulene	1503		0.9 $\pm$ 0.05		0.2				
(E,E)- $\alpha$ -Farnesene	1506	1700	0.8 $\pm$ 0.01		tr				
$\beta$ -Bisabolene	1506	1743		0.4 $\pm$ 0.01		0.3			
$\gamma$ -Cadinene	1514		1.8 $\pm$ 0.22	0.4 $\pm$ 0.01	0.3	1.5	tr		
(Z)- $\gamma$ -Bisabolene	1515			0.2 $\pm$ 0.01		0.2			
6-Methyl- $\alpha$ -ionone	1522		2.23 $\pm$ 0.07		tr				
7- <i>epi</i> - $\alpha$ -Selinene	1522			0.1 $\pm$ 0.00		1.5			
$\delta$ -Cadinene	1523	1764		0.9 $\pm$ 0.04	0.6	0.1			
(E)- $\gamma$ -Bisabolene	1531			0.3 $\pm$ 0.02		tr			
$\gamma$ -Dihydro- <i>ar</i> -himachalene	1532			0.2 $\pm$ 0.02		tr			
<i>cis</i> -Calamenene	1540			0.2 $\pm$ 0.02		0.01			
Germacrene B	1561	1544	4.9 $\pm$ 0.08		0.7				
(E)-Nerolidol	1563	1997	0.2 $\pm$ 0.00	1.8 $\pm$ 0.21	tr	tr			
Spathulenol	1578	1250	0.6 $\pm$ 0.02	tr	tr				
Caryophyllene oxide	1583	2071	0.4 $\pm$ 0.01	5.1 $\pm$ 0.34	tr	0.5			
Globulol	1585		0.2 $\pm$ 0.04						
(E)-2-Hexenyl benzoate	1588			1.3 $\pm$ 0.13					
Viridiflorol	1593			tr					
Salvial-4(14)-en-1-one	1595		2.8 $\pm$ 0.05		0.11				
Unknown	1598			1.2 $\pm$ 0.08		tr			
<i>n</i> -Hexadecane	1600		t						
Unknown	1602			0.5 $\pm$ 0.05					
5- <i>epi</i> -7- <i>epi</i> - $\alpha$ -Eudesmol	1608			0.2 $\pm$ 0.01					
Humulene epoxide II	1608	2072	0.8 $\pm$ 0.02	1.6 $\pm$ 0.15		tr			
1,10-di- <i>epi</i> -Cubanol	1619		0.8 $\pm$ 0.01	0.5 $\pm$ 0.07	0.13				
10- <i>epi</i> - $\gamma$ -Eudesmol	1624		1.9 $\pm$ 0.08	0.3 $\pm$ 0.03					
1- <i>epi</i> -Cubanol	1629		6.4 $\pm$ 0.07	0.3 $\pm$ 0.03	tr				
$\beta$ -Cedren-9-one	1631		0.3 $\pm$ 0.05						
$\gamma$ -Eudesmol	1632	2183		0.3 $\pm$ 0.03					
<i>epi</i> - $\alpha$ -Cadinol	1640		3.7 $\pm$ 0.22		tr				
Caryophylla-4(14),8(15)-dien-5 $\alpha$ / $\beta$ -ol	1641			0.5 $\pm$ 0.07					
$\tau$ -Cadinol	1646	2158		0.7 $\pm$ 0.09		tr			
$\alpha$ -Muurolole	1646	2209	1.4 $\pm$ 0.09	0.2 $\pm$ 0.02	tr				
$\beta$ -Eudesmol	1651	2256		0.3 $\pm$ 0.04					
$\alpha$ -Cadinol	1654	2235	7.4 $\pm$ 0.23	1.0 $\pm$ 0.07	tr				
3-Thujopsanone	1655			0.6 $\pm$ 0.07		0.01			
Patchuli alcohol	1658			1.2 $\pm$ 0.14					
5- <i>iso</i> -Cedranol	1674			0.2 $\pm$ 0.01					
<i>epi</i> - $\beta$ -Bisabolol	1675	2215		3.9 $\pm$ 0.26		tr			
Khumisol	1680		4.5 $\pm$ 0.05		tr				
<i>epi</i> - $\alpha$ -Bisabolol	1685	2219		1.6 $\pm$ 0.11		tr			
Acorenone	1693			0.7 $\pm$ 0.03					
<i>n</i> -Heptadecane	1700		1.0 $\pm$ 0.07	0.8 $\pm$ 0.02	tr				
<b>total</b>			<b>91.4 <math>\pm</math> 0.86</b>	<b>95.8 <math>\pm</math> 1.25</b>	<b>99.9</b>	<b>92.8</b>	<b>98.5</b>	<b>99.9</b>	

<sup>a</sup>Linear Retention Indices (HP-5 Column); <sup>b</sup>Linear Retention Indices (HP-WAX Column)

**Table 2:** Chemical composition of the leaf and root EOs and the HS of the leaves, roots, fruits and seeds of *C. angolense*.

	Leaf EO	Root EO	Leaf HS	Root EO	Fruit EO	Seed EO
Hydr. Monoterpenes	7.5	3.0	38.2	2.8	93.3	90.8
Oxyg. Monoterpenes	2.3	21.4	0.3	1.0	1.5	4.1
<b>Tot. Monoterpenes</b>	<b>9.8</b>	<b>24.4</b>	<b>38.5</b>	<b>3.8</b>	<b>94.8</b>	<b>94.9</b>
Hydr. Sesquiterpenes	37.5	31.8	49.0	93.0	0.6	0.6
Oxyg. Sesquiterpenes	31.2	20.7	0.2	0.7		
<b>Tot. Sesquiterpenes</b>	<b>68.8</b>	<b>52.5</b>	<b>49.2</b>	<b>93.7</b>	<b>0.6</b>	<b>0.6</b>
Hydrocarbons	10.7	0.8	5.0		3.3	3.3
Oxyg. Hydrocarbons	2.1	11.1		1.4		
<b>Tot. Hydrocarbons</b>	<b>12.8</b>	<b>11.9</b>	<b>5.0</b>	<b>1.4</b>	<b>3.3</b>	<b>3.3</b>

or hydrocarbons were quantitatively quite dissimilar. The total amounts of monoterpenes and hydrocarbons were quite similar in both the EOs, with oxygenated compounds prevalent in the root EO (34.6% in comparison with 4.4% of the leaves) and *vice versa* in the case of non-oxygenated compounds (5.4% in the roots and 17.8% in the leaves).  $\beta$ -Caryophyllene (19.7%) and its derivatives characterized the chemical profile of the root EO, while the leaf oil did not show a prevalent compound, but the aroma was composed of a pool of 7 constituents at percentages ranging from 4.5 to 9.3 (Table 1). The volatile composition of EOs obtained from leaves and roots of *C. panchonii*, *C. tinctorium* and *C. vitifolium* are

reported in the literature [1a,3,4,6b,7]. These data gave a high percentage of sesquiterpenes in *C. panchonii* (80%) and *C. vitifolium* (75%) leaf EO, similar to the results reported here, while *C. tinctorium* was characterized by a major presence of oxygenated aliphatic compounds (40%). With regard to the results obtained from *C. angolense* roots, the chemical composition of the EO was completely different from data reported for *C. tinctorium* and *C. panchonii*, since a terpenoidic fraction was not detected. On the contrary, data from *C. vitifolium* roots showed a high percentage of terpenoids, in agreement with our results.

As expected, the headspace analysis of the leaves and roots showed quantitative differences in their chemical profile with respect to the corresponding EOs. In the leaf HS and in the EO analysis, total sesquiterpenes were the main chemical group, but their percentages considerably differed (49.2 compared to 68.8% in EO). In the case of total monoterpenes, their amounts in HS were higher than in the corresponding EO (especially oxygenated monoterpenes). A total 93.9% of sesquiterpenes were present in the root HS, while the same class of constituents reached only 53.2% in the EO from the same plant organs. At the same time, in the root HS, the percentage of monoterpenes decreased dramatically (from 26.2 to

3.7%).  $\beta$ -Caryophyllene and germacrene D were the main constituents of root and leaf HS, respectively, but in higher percentages (57.3% and 22.8%, respectively) than in the corresponding EOs.

Regarding the volatile composition of the fruits and seeds of *C. angolense*, a different profile was evidenced. HS analyses were characterized by a high percentage of monoterpenes (94.6% seeds and 95.1% fruits) with respect to the other HS analyzed. In fact, sesquiterpenes represented almost the entire amount in the HS from the roots, while sesquiterpenes and monoterpenes showed comparable percentages in leaf HS (49.2% and 38.5%, respectively).

Data obtained from the analysis of the volatile fractions of *C. angolense* aerial parts showed that monoterpenes are mostly emitted in the atmosphere (76.1%, total obtained by averaging the values of HS of leaves, fruits and seeds), in good agreement with the results reported by Chen [5]. This high production of monoterpene derivatives could represent a defense mechanism of the plant against a hostile environment characterized by a very high air temperature and long periods of exposure to sunlight, typical of the tropical areas where *C. angolense* grows. This result is more evident if we consider the total absence of these compounds in the roots, where sesquiterpenes are produced in abundance, probably to preserve roots from pathogen attacks or as allelochemicals [6a]. This paper represents the first study on the volatile constituents of different organs of *C. angolense*.

## Experimental

**Plant materials:** Leaves, roots, fruits and seeds of *Cochlospermum angolense* were collected from the Huambo area, Angola (Planalto Central, altitude between 1520 and 1830 m) and authenticated by Sister Manuela Salvadori, a member of the Trappist community of Angola. The samples were collected in April 2010. A voucher specimen is deposited at the Dipartimento di Scienze Farmaceutiche (Pisa, Italy).

**Isolation of essential oils:** Air-dried plant material (100 g) from leaves or roots of *C. angolense* was subjected to hydro-distillation for 2 h in a Clevenger-type apparatus. The essential oils collected

over water, were dried over anhydrous sodium sulfate and preserved in a refrigerator until analysis.

**Head-space solid-phase microextraction (HS-SPME):** A SPME fiber with a 100  $\mu$ m layer of polydimethylsiloxane (PDMS) was used for sampling the volatile constituents in the headspace. Leaves and roots (1 g) were placed separately in 5 mL glass vials. The SPME fiber was exposed in the headspace of the vial at room temperature for 10 mins, then it was removed from the vial and introduced into the GC injector. Three mins thermal desorption was carried out.

**GC and GC/MS analysis:** GC analyses were accomplished with an HP-5890 Series II instrument equipped with a HP-Wax and HP-5 capillary columns (both 30 m X 0.25 mm, 0.25  $\mu$ m film thickness), working with the following temperature program: 60°C for 10 min, rising at 5°C/min to 220°C. The injector and detector temperatures were maintained at 250°C; carrier gas, nitrogen (2 mL/min); detector, dual FID; split ratio 1:30. The volume injected was 0.5  $\mu$ L. The relative proportions of the oil constituents were percentages obtained by FID peak-area normalization without the use of a response factor. GC-MS analyses were performed with a Varian CP3800 gas chromatograph equipped with a DB-5 capillary column (30 m X 0.25; coating thickness, 0.25  $\mu$ m) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions were as follows: injector and transfer line temperature, 220 and 240°C at 3°C, respectively; oven temperature, programmed from 60 to 240°C at 3°C min; carrier gas, helium at 1 mL/min; injection, 0.2  $\mu$ L (10% *n*-hexane solution); split ratio, 1:30.

**Compound identification:** Identification of the constituents was based on comparison of the retention time with those of authentic samples, comparing their linear indices relative to a series of *n*-hydrocarbons, and on computer matching against commercial (NIST 98 and ADAMS) and a homemade library of mass spectra built up from pure substances and components of known oils, and MS literature data [8-11].

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