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Volatiles from Steam-distilled Leaves of Some Plant Species from Madagascar and New Zealand and Evaluation of Their Biological Activity

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Steam-distilled aerial parts of *Ravensara aromatica* and *Cinnamomum camphora* from Madagascar and *Leptospermum scoparium* from New Zealand have been subjected to qualitative and quantitative analysis by means of GC techniques. This allowed the elucidation of conflicting data present in the available literature for these species. Also, the biological activity *in vitro* was evaluated by measuring MICs and GIZs.

Keywords: *Ravensara aromatica* Sonn., *Cinnamomum camphora* L., *Leptospermum scoparium* J.R. Forst. & G. Forst., Madagascar, New Zealand, quantitative analysis, antimicrobial.

Ravensara aromatica Sonn. is an evergreen tree belonging to the family Lauraceae. It is also reported as *R. anisata* Danguy, although sometimes it has been considered as a separate species [1a,1b]. The leaves, when subjected to distillation, give a colorless to light yellow oil, which smells like eucalyptus. One of the earliest studies of *R. aromatica* describes the composition of a series of essential oils (not only *R. aromatica*) analyzed by means of GC-MS and MDGC [1c]. The results obtained were in contrast with others published later [1d], which again considered *R. aromatica* and *R. anisata* as two different species with different major constituents in their essential oils. According to a later paper [2], some of the conclusions published [1b] seem to be problematic, as the most characteristic constituents of *R. aromatica* were confirmed as methyl chavicol and methyl eugenol, as already reported by Möllenbeck *et al*. [1c]. At the same time, another study aimed to bring clarity to the uncertainty of data presented in the past on *R. aromatica* [3a]. By means of hierarchical clustering analysis, applied to new samples harvested in different zones of Madagascar, the authors established chemical markers for four different groups, which correlated with the literature data. These data are in agreement with another brief report from the same year [3b].

C. camphora L. from Madagascar has been scarcely investigated up to now. In the literature, there is a report on the species from Kenya which can be related to the Madagascan oils [4a], although the results presented seem problematic. In the same study reported above for *R. aromatica* [2], samples of *C. camphora* from Madagascar presented 1,8-cineole, α-terpineol and sabinene as major components. Another paper [4b] about Ravintsara, which is the Madagascan name for *C. camphora*, reports the GC composition of the oil and its possible therapeutic applications.

A completely different ecosystem can be found in New Zealand, where *Leptospermum scoparium* J.R. et G. Forst (family Myrtaceae) has its own habitat. This shrub, that can reach the dimensions of a tree, is sometimes confused with *Melaleuca alternifolia*, commonly known as tea tree, because of the similar use made traditionally of these two species. However, the morphology of tea tree is quite different from that of *L. scoparium*, which is better known as manuka.

Table 1: GC composition of the three oils analyzed. R.F.: Response Factor. LRI_{exp} : Experimental Linear Retention Index on SLB-5MS column. LRI_{lib}: LRI reported in the libraries utilized for spectral matching; tr = ≤0.05.

Compound	**R.F.**	**LRI_{exp}**	**LRI_{lib}**	**Ravensara**		**Leptospermum**		**Cinnamomum**	
				% area	**g/100**	**% area**	**g/100 g**	**% area**	**g/100 g**
Tricyclene	*1.0*	**923**	**921**	tr	tr	-	-	-	-
α-Thujene	*1.0*	**925**	**927**	0.6	0.7	tr	tr	tr	tr
α-Pinene	*1.0*	**933**	**933**	3.0	3.2	1.8	1.8	1.5	1.3
α-Fenchene	*1.0*	**948**	**948**	tr	tr	-	-	-	-
Camphene	*1.0*	**949**	**953**	0.6	0.6	-	-	tr	tr
Sabinene	*1.0*	**972**	**972**	4.5	4.7	tr	tr	2.2	1.9
β-Pinene	*1.0*	**978**	**978**	2.1	2.2	0.1	0.1	0.3	0.2
3-Octanone	*1.3*	**983**	**979**	-	-	-	-	0.1	0.2
Myrcene	*1.0*	**988**	**991**	2.3	2.4	0.3	0.3	0.6	0.5
6-Methyl-5-hepten-2-ol	*1.3*	**993**	**979**	-	-	-	-	0.1	0.1
Ethyl hexanol	*1.3*	**997**	**999**	-	-	-	-	tr	tr
Isopentyl isobutyrate	*1.6*	**1010**	**1007**	-	-	tr	tr	-	-
α-Phellandrene	*1.0*	**1007**	**1007**	1.2	1.3	-	-	0.2	0.1
δ-3-Carene	*1.0*	**1010**	**1009**	2.6	2.7	-	-	-	-
α-Terpinene	*1.0*	**1018**	**1018**	9.4	9.8	tr	tr	0.1	0.1
p-Cymene	*1.0*	**1025**	**1025**	1.5	1.6	0.1	0.1	8.2	7.3
Limonene	*1.0*	**1031**	**1030**	27.5	28.8	0.1	0.1	38.6	34.1
(Z)-β-Ocimene	*1.0*	**1035**	**1032**	1.0	1.1	-	-	-	-
1,8-Cineole	*1.3*	**1036**	**1032**	-	-	0.1	0.2	43.1	49.5
Heptyl acetate	*1.6*	**1037**	**1038**	0.1	0.2	-	-	-	-
(E)-β-Ocimene	*1.0*	**1045**	**1046**	0.3	0.3	tr	tr	0.9	0.8
γ-Terpinene	*1.0*	**1058**	**1058**	2.9	3.0	0.1	0.1	0.8	0.7
cis-Sabinene hydrate	*1.3*	**1070**	**1069**	tr	tr	-	-	tr	tr
Terpinolene	*1.0*	**1086**	**1086**	0.3	0.3	tr	tr	0.1	0.1
p-Cymenene	*1.0*	**1090**	**1091**	-	-	-	-	tr	tr
Linalool	*1.3*	**1101**	**1101**	6.5	8.9	0.1	0.1	0.2	0.2
Isoamyl isovalerate	*1.6*	**1104**	**1109**	-	-	0.1	0.1	tr	tr
2-Methylbutyl isovalerate	*1.6*	**1107**	**1103**	-	-	tr	tr	-	-
3-Methyl-, 3-butenyl-3-methyl butyrate	*1.6*	**1114**	**1114**	-	-	0.1	0.2	-	-
Fenchyl alcohol	*1.3*	**1119**	**1114**	tr	tr	-	-	tr	tr
trans-p-Mentha-2,8-dien-1-ol	*1.3*	**1121**	**1122**	-	-	-	-	tr	tr
cis-p-Menth-2-en-1-ol	*1.3*	**1125**	**1124**	tr	tr	-	-	tr	tr
Allocimene	*1.0*	**1128**	**1132**	tr	tr	-	-	0.1	0.1
cis-Limonene oxide	*1.5*	**1133**	**1134**	-	-	-	-	tr	tr
cis-p-Mentha-2,8-dien-1-ol	*1.3*	**1136**	**1138**	-	-	-	-	tr	tr
trans-Pinocarveol	*1.3*	**1141**	**1141**	-	-	-	-	0.1	0.1
trans-p-Menth-2-en-1-ol	*1.3*	**1142**	**1141**	tr	tr	-	-	-	-
Camphor	*1.3*	**1147**	**1149**	-	-	-	-	tr	tr
Menthone	*1.3*	**1155**	**1158**	-	-	-	-	tr	tr
Menthofuran	*1.9*	**1164**	**1159**	-	-	-	-	tr	tr
Borneol	*1.3*	**1173**	**1173**	0.1	0.1	-	-	tr	tr
Menthol	*1.3*	**1177**	**1184**	-	-	-	-	0.1	0.1
Terpinen-4-ol	*1.3*	**1182**	**1177**	2.0	2.8	tr	tr	0.2	0.2
Cryptone	*1.3*	**1187**	**1187**	-	-	-	-	tr	tr
Isoamyl tiglate	*1.6*	**1193**	**1196**	-	-	tr	0.1	-	-
α-Terpineol	*1.3*	**1196**	**1195**	0.2	0.3	tr	tr	0.2	0.2
trans-Carveol	*1.3*	**1219**	**1215**	-	-	-	-	tr	tr
(2E)-Decenal	*1.3*	**1262**	**1265**	-	-	tr	tr	-	-
Estragole	*1.3*	**1200**	**1196**	7.2	9.9	-	-	-	-
trans-Piperitol	*1.3*	**1210**	**1209**	tr	tr	-	-	-	-
Pulegone	*1.3*	**1238**	**1237**	-	-	-	-	tr	tr
Carvone	*1.3*	**1244**	**1243**	-	-	-	-	tr	tr
Bornyl acetate	*1.6*	**1285**	**1285**	tr	0.1	-	-	-	-
(E)-Anethole	*1.3*	**1287**	**1285**	0.2	0.2	-	-	-	-
Menthyl acetate	*1.6*	**1289**	**1290**	-	-	-	-	tr	tr
Carvacrol	*1.3*	**1299**	**1300**	-	-	-	-	tr	tr
Bicycloelemene	*1.0*	**1334**	**1338**	-	-	0.1	0.1	-	-
δ-Elemene	*1.0*	**1337**	**1335**	0.1	0.1	tr	tr	tr	tr
α-Terpinyl acetate	*1.6*	**1347**	**1349**	tr	tr	-	-	tr	tr
α-Cubebene	*1.0*	**1349**	**1349**	0.1	0.1	2.2	2.2	-	-
Eugenol	*1.3*	**1353**	**1357**	tr	tr	-	-	-	-
Cyclosativene	*1.0*	**1371**	**1367**	tr	tr	0.3	0.3	-	-
α-Copaene	*1.0*	**1379**	**1374**	3.0	3.2	36.0	35.9	tr	tr
β-Bourbonene	*1.0*	**1386**	**1387**	tr	tr	-	-	0.1	0.1
β-Cubebene	*1.0*	**1390**	**1392**	0.2	0.2	2.9	2.9	-	-
β-Elemene	*1.0*	**1391**	**1391**	0.1	0.1	-	-	tr	tr
Benzyl isovalerate	*1.6*	**1394**	**1399**	-	-	0.1	0.1	-	-
Methyl eugenol	*1.3*	**1403**	**1404**	2.7	3.7	-	-	-	-
Cyperene	*1.0*	**1406**	**1407**	-	-	0.1	0.1	-	-
α-Gurjunene	*1.0*	**1410**	**1413**	tr	tr	0.5	0.5	-	-
α-cis-Bergamotene	*1.0*	**1416**	**1413**	tr	tr	-	-	-	-
β-Maaliene	*1.0*	**1417**	**1414**	-	-	0.1	0.1	-	-
(E)-Caryophyllene	*1.0*	**1424**	**1424**	3.8	4.0	13.1	13.1	0.6	0.5

Calarene	*1.0*	**1433**	**1432**	tr	tr	-	-	-	-
β-Copaene	*1.0*	**1433**	**1432**	-	-	0.1	0.1	tr	tr
α-*trans* -Bergamotene	*1.0*	**1435**	**1434**	tr	tr	tr	tr	tr	tr
α-Maaliene	*1.0*	**1438**	**1438**	-	-	tr	tr	-	-
Aromadendrene	*1.0*	**1442**	**1438**	tr	tr	0.5	0.5	-	-
3,5-Cadinadiene	*1.0*	**1453**	**1452**	-	-	4.2	4.2	-	-
α-Humulene	*1.0*	**1459**	**1454**	0.4	0.4	1.9	1.9	tr	tr
9-*epi-(E)*-Caryophyllene	*1.0*	**1463**	**1464**	0.1	0.1	1.1	1.1	tr	tr
cis-Muurola-4(14),5-diene	*1.0*	**1474**	**1465**	-	-	-	-	tr	tr
1(6),4-Cadinadiene	*1.0*	**1475**	**1472**	tr	tr	1.3	1.3	-	-
γ-Muurolene	*1.0*	**1478**	**1478**	0.1	0.1	0.1	0.1	tr	tr
Germacrene D	*1.0*	**1484**	**1479**	0.8	0.8	0.9	0.9	0.1	0.6
β-Selinene	*1.0*	**1492**	**1492**	tr	0.1	1.2	1.2	-	-
γ-Amorphene	*1.0*	**1495**	**1490**	-	-	0.9	0.9	-	-
Bicyclogermacrene	*1.0*	**1499**	**1494**	0.1	0.2	-	-	0.1	0.1
α-Selinene	*1.0*	**1500**	**1501**	-	-	1.8	1.8	-	-
α-Muurolene	*1.0*	**1501**	**1500**	0.1	0.1	0.6	0.6	tr	tr
(E,E)-α-Farnesene	*1.0*	**1504**	**1504**	-	-	0.4	0.4	0.1	0.1
α-Bulnesene	*1.0*	**1505**	**1505**	tr	tr	-	-	-	-
β-Bisabolene	*1.0*	**1509**	**1508**	0.1	0.1	-	-	tr	tr
γ-Cadinene	*1.0*	**1516**	**1512**	tr	tr	0.2	0.2	tr	tr
Cubebol	*1.3*	**1518**	**1519**	tr	tr	-	-	-	-
δ-Cadinene	*1.0*	**1522**	**1518**	0.4	0.5	5.8	5.8	0.1	0.1
trans-Calamenene + Zonarene	*1.0*	**1525/1526**	**1527/1528**	0.1	0.1	5.7	5.6	-	-
(E)-γ-Bisabolene	*1.0*	**1529**	**1529**	tr	tr	-	-	-	-
Naphthalene 1,2,3,4,4a,7-hexahydro-,1,6-dimethyl-,4-(1-methylethyl)-, (1α, 4β , 4aβ)-	*1.0*	**1535**	**1536**	tr	tr	2.0	1.9	-	-
Flavesone	*1.3*	**1539**	**1539**	-	-	1.1	1.4	-	-
α-Cadinene	*1.0*	**1540**	**1537**	-	-	-	-	tr	tr
α-Calacorene	*1.0*	**1545**	**1544**	-	-	0.4	0.4	-	-
Elemicin	*1.3*	**1547**	**1551**	tr	tr	-	-	-	-
α-Elemol	*1.3*	**1550**	**1546**	0.1	0.1	-	-	-	-
(E)-Nerolidol	*1.3*	**1561**	**1561**	-	-	tr	tr	-	-
Germacrene B	*1.0*	**1563**	**1559**	tr	tr	-	-	-	-
β-Calacorene	*1.0*	**1566**	**1564**	-	-	0.1	0.1	-	-
Ledol	*1.3*	**1574**	**1574**	-	-	0.1	0.1	-	-
Spathulenol	*1.3*	**1580**	**1576**	-	-	0.2	0.3	tr	tr
Caryophyllene oxide	*1.5*	**1586**	**1587**	0.1	0.1	0.3	0.5	tr	tr
Viridiflorol	*1.3*	**1597**	**1594**	-	-	0.1	0.1	tr	tr
Guaiol	*1.3*	**1599**	**1593**	tr	tr	-	-	-	-
Humulene epoxide	*1.5*	**1613**	**1613**	tr	tr	-	-	-	-
Isoleptospermone	*1.3*	**1614**	**1621**	-	-	1.2	1.6	-	-
Leptospermone	*1.3*	**1625**	**1629**	-	-	4.8	6.3	-	-
Epicubenol	*1.3*	**1632**	**1627**	tr	tr	0.4	0.6	-	-
γ-Eudesmol	*1.3*	**1636**	**1632**	tr	tr	-	-	-	-
Bicyclo(7.2.0)undecan-3-ol, 11,11-dimethyl-, 4,8-bis(methylene)-	*1.3*	**1643**	**1636**	-	-	0.1	0.1	-	-
T-Muurolol	*1.3*	**1647**	**1640**	tr	tr	-	-	-	-
Cubenol	*1.3*	**1648**	**1649**	-	-	0.5	0.7	-	-
α-Muurolol	*1.3*	**1650**	**1651**	tr	tr	0.1	0.2	-	-
4-Cadinen-10-ol	*1.3*	**1660**	**1659**	-	-	0.1	0.2	-	-
Selin-11-en-4-α-ol	*1.3*	**1663**	**1658**	-	-	0.1	0.1	-	-
TOTAL				**89.4**	**100.0**	**97.2**	**100.0**	**99.8**	**100.0**

Table 2: Aromatogram showing the growth inhibition zones (diameters) of the three oils analyzed and of some constituents.

	Ps. aer.	**Es. coli**	**Ba. sub.**	**Sta.aur.**	**Pro. mir.**	**Ser. mar.**	**Kl. pne.**	**Ps. cepa.**	**Cand.alb.**	**Asp. nig.**
Ra.ara.	-	-	12 mm	22 mm	13 mm	pigm. tr.	9 mm	-	14 mm	No black pigm.
Ci.cam.	-	-	19 mm	23 mm	-	-	9 mm	-	9 mm	No black pigm.
Lep.sc.	-	-	25 mm	18 mm	-	13 mm pigm.	-	-	30 mm	No black pigm.
α-terp.	-	-	15 mm	13 mm	-	10 mm pigm.	-	-	-	No black pigm.
Ter-4-ol	-	-	21 mm	18 mm	32 mm	23 mm no pigm.	25 mm	-	18 mm	No black pigm.
Estr.	-	8 mm	20 mm	22 mm	11 mm	10 mm no pigm.	8 mm	-	19 mm	12-13 mm no black pigm.
p-Cym.	-	-	-	-	-	pigm.	-	-	-	No black pigm.
γ-Terp.	-	-	-	-	-	-	-	-	9 mm	Pigm.
α-cop.	-	-	11 mm	-	-	-	-	-	11 mm	Pigm.
(E)-car.	-	-	21 mm	-	-	-	-	-	-	Pigm.
Sab.	-	-	13 mm	14.5 mm	-	-	-	-	11 mm	Pigm.
Lim.	-	-	10-11 mm	12 mm	-	-	-	-	10 mm	Pigm.
α-Pin.(+)	-	-	10 mm	-	-	-	-	-	11 mm	Pigm.
β-Pin.	-	-	9 mm	8 mm	-	-	-	-	10 mm	Pigm.
Euc.	-	8 mm	9 mm	8 mm	8 mm	9 mm pigm. tr.	-	-	14 mm	Pigm.
α-Pin.(-)	-	-	10 mm	-	-	-	-	-	10 mm	Pigm.

Ra.ara.= *R.aromatica*; Ci.cam.= *C.camphora*; Lep.sc.= *L.scoparium*; α-Terp.=α-terpineol; Ter-4-ol=Terpinen-4-ol; Estr.=estragole; *p*-Cym.=*p*-cymene; γ-terp.=γ-terpinene; α-cop.=α-copaene; (*E*)-car.=(*E*)-caryophyllene; Sab.=sabinene; Lim.=limonene; α-Pin(+)=α-pinene; β-pin.=β-pinene; Euc.=eucalyptol; α-Pin.(-)=α-pinene(-); pigm.=pigment; pigm.tr.=pigment traces.

Table 3: MIC values (mg/mL) of the three oils and three reference standards.

	R. aromatica	*L. scoparium*	*C. camphora*	*Terpinen-4-ol*	*Estragole*	*α-terpinene*
B. subtilis	0.0089	0.0089	0.089	0.0089	0.0089	0.0089
S. aureus	0.089	0.0089	0.089	0.089	0.00089	0.089
S. marcescens	0.089	0	0.089	0.0089	0.089	0.089
C. albicans	0.089	0.089	0.089	0.0089	0.0089	0.089
A. niger	0.089	0	0.089	0.0089	0.089	0.089

L. scoparium essential oil production is generally concentrated in a specific area of the North Island, which spreads out from the Coromandel peninsula to the East Cape. Previous works have demonstrated that the chemical composition of manuka oil changes considerably depending upon the zone of harvest [5a,5b,6a,6b]. In particular, analyses from 261 individual manuka plants collected from 87 sites in New Zealand revealed the existence of several chemotypes with >20% triketone content [6c]. The traditional use of manuka oil as a medicine among Maori people has excited the interest of the scientific community. From ethnomedicine we know that manuka was employed in urinary and intestinal pathologies, sucking the gum for coughs, inhaling the vapour for colds, as poultices for skin damage, and applying the gum to drain excess body liquids [6d].

In this study the detailed chemical composition (weight % and peak areas) and antimicrobial activity of *R. aromatica*, *C. camphora* and *L. scoparium* steam-distilled essential oils have been investigated. In particular some commercially available samples and their components have been tested against a series of microrganisms. Accurate GC-FID and GC-MS analyses, with the support of an area normalization procedure, have led to the quantitative and qualitative determination of components, some of them previously unreported.

Table 1 reports the qualitative and quantitative composition of the components determined in the three species analyzed. The constituents are listed in order of elution from the SLB-5MS column. In total, 123 compounds were determined. Predominant classes of components were: sesquiterpene hydrocarbons in *L. scoparium*, with α-copaene (35.9 g/100 g), *(E)*-caryophyllene (13.1 g/100 g), and others; the monoterpenoid cyclic ether 1,8-cineole was the highest constituent of *C. camphora* (49.5 g/100g), followed by limonene (34.1 g/100 g); monoterpene hydrocarbons were predominant in *R. aromatica* with limonene (28.8 g/100 g), sabinene (4.7 g/100 g), and others. With *R. aromatica*, the quali-quantitative composition determined in this study closely matches that reported by Andrianoelisoa *et al*. [3a]: major constituents were limonene (27.5%), α-terpinene (9.4%), methyl chavicol (7.2%), linalool (6.5%), sabinene (4.5%) and methyl eugenol (2.7%). Holm *et al*. [1d] reported 1,8-cineol as the predominant component in *R. aromatica*, probably mistaking *R. anisata* with *R. aromatica*. *R. aromatica* and *C. camphora* presented quantitative similarities for some compounds, such as limonene, sabinene and α-pinene. Noteworthy are the presence in *L. scoparium* of the β-triketones flavesone (1.1%), leptospermone (4.8%) and isoleptospermone (1.2%), reported in the literature for this plant species [6a] and considered bioactive components. In fact, several papers have emphasized the antibacterial activity of manuka honey [6d,7a] and the antifungal [7b], cytotoxic [7c], antibacterial [8a], acaricidal [8b], herbicidal [9a] and spasmolytic [9b] actions of the essential oil, attributed to the presence of these β-triketones.Table 2 reports the results relative to the aromatogram. The most significant activity was detected against *B. subtilis*, *S. aureus* and *S. marcescens*, and against *C. albicans* and *A. niger*.

In particular, *L. scoparium* was the most effective against *B. subtilis* (25 mm i.z.), while the other two oils were more effective on *S. aureus* (23 and 22 mm). *S. marcescens* underwent pigment inhibition when inoculated with *R. aromatica* and with the standards estragole and terpinen-4-ol. *C. albicans* was inhibited by *L. scoparium*, terpinen-4-ol and estragole, while *A. niger* underwent only pigment inhibition. MICs (Table 3) were higher (0.089 mg/mL) for *C. camphora* against *B. subtilis*, and for *R. aromatica* and *C. camphora* against *S. aureus*. Very low MIC values were observed for *L. scoparium* against *S. aureus*, along with α-terpinene and terpinen-4-ol. *S. marcescens* was inhibited by terpinen-4-ol at a concentration of 0.0089 mg/mL. The lowest MIC toward *C. albicans* was 0.0089 mg/mL, shown by terpinen-4-ol and estragole. Finally, as concerns *A. niger*, MIC values were 0.089 mg/mL except for *L. scoparium* that did not inhibit the growth; the lowest MIC observed was for terpinen-4-ol (0.0089 mg/mL).

Experimental

Samples: Essential oils of *Leptospermum scoparium*, *Ravensara aromatica* and *Cinnamomum camphora* were supplied by EOU (Essential Oil University), USA. The genuineness of the oils was certified by PhD chemist dr. Robert Pappas, who is responsible for the company management; the oils were directly imported from the growers.

Analysis: GC-FID analyses were carried out by means of a GC-2010 (Shimadzu) equipped with a 30 m x 0.25 mm I.D. x 0.25 μm d_f SLB-5MS column (Supelco). Oven temperature program was from 50°C to 250°C at 3°C/min, held 5 min. Injection took place in split mode, with a split ratio of 50:1; volume injected was 1.0 μL (oils diluted 1:10 in *n*-hexane). Both injector and FID temperatures were set at 280°C. Carrier gas used was helium, at 30.1 cm/s and a pressure of 99.8 kPa. Data were processed by *GCsolution* software (Shimadzu). GC/MS analyses were carried out with a GCMS-QP2010 system (Shimadzu). GC parameters were the same as those used for GC analysis. MS conditions were: Temperatures of ion source and interface 200°C and 250°C, respectively; scan interval 0.25 s and mass range 40-400 m/z. Data handling was performed by the software *GCMSsolution ver.2.5* (Shimadzu).

The GC-MS system was provided with commercial databases containing reference spectra coming from natural products, flavors and fragrances [9c,10,11]. Quantitative analysis was carried out by means of the internal standard method along with area normalization with response factors. The procedure utilized has been consolidated by previous work [12,13], here briefly reported for better comprehension of the results obtained. In particular, the following equation was utilized:

$$C\% = 100\ A_x\ f_x / \Sigma\ A\ f$$

where C is analyte concentration, A_x is analyte peak area, f_x is its response factor and Σ A f is the summation of the products of each analyte peak area for the relative response factor. In their turn, response factors were measured based on chemical groups, in consideration of the unavailability of all the components determined as commercial reference standards. Therefore, one or more chemical standards for various chemical groups (alcohols, monoterpenes, sesquiterpenes, ketones, furans) were chosen, then injected at 5 different concentrations, 5 times for each concentration. This allowed the build up of a 5 point calibration curve. Each value referred to nonane, as internal standard, which was added to each solution up to a final concentration of 10.05 g/100 mL. For a better comparison with published data, % composition was also reported coming from the raw peak areas of chromatograms.

Antimicrobial activity: The whole oils and some standard compounds (present as constituents of the oils) were tested by means of the aromatogram technique against 10 microrganisms. The aromatogram is a test for antibacterial activity of essential oils in which the oil is introduced into a Petri dish containing bacteria. A clear zone indicates the bactericidal activity of the oil [14].

Standards were α-terpinene, terpinen-4-ol, methyl chavicol, *p*-cymene, γ-terpinene, α-copaene, (*E*)-caryophyllene, sabinene, limonene, (+)-α-pinene, β-pinene, 1,8-cineole, (-)-α-pinene. Standards were chosen on the basis of both market availability and quantity of the analyte in the original matrix. Eight stocks were used for testing: *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Proteus mirabilis*, *Serratia marcescens*, *Klebsiella pneumoniae*, and *Pseudomonas cepacia*. Two mycetes were also used: *Candida albicans* and *Aspergillus niger*. Oils and standards brought to room temperature were tested for antibiosis using the following culture media: Mueller Hinton Agar (MHA) for bacterial stocks, and Sabouraud Dextrose Agar (SDA) for mycetes. Each stock was inoculated in Triptyc Soy Broth (TSB) and thermostatted for 18-24 hours at 37°C. Mycetes were sown in SDA and kept at 30°C for 2-5 days. Growth inhibition (Kirby-Bauer method) was evaluated after incubation at 37°C for 24 h of the agar media inoculated with germs and covered with 10 μL of each oil/standard. Candida and Aspergillus were incubated at 30°C for 24-48 h and 5 days, respectively. The zones of inhibition were then measured in mm. The Minimum Inhibitory Concentration (MIC) was also evaluated by incremental dilution of oil samples and standards within the range $10^{-1} – 10^{-4}$.

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