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INFRASPECIFIC TAXONOMY AND ESSENTIAL OIL CHEMOTYPES IN SWEET BASIL, *OCIMUM BASILICUM*

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Key Word Index—*Ocimum basilicum*; *Ocimum* × *citriodorum*; Lamiaceae; basil; essential oils; chemotypes; chemotaxonomy.

Abstract—Essential oil compositions of fresh and freeze-dried leaves were determined for 16 accessions of *Ocimum basilicum* belonging to different varieties to see whether they could be used as infraspecific taxonomic characters. One accession of *O.* × *citriodorum* was also studied. Some 30 monoterpenoids, sesquiterpenoids and phenylpropanoids were identified, the major components (more than 20% of the total essential oil composition in one or more accessions) being geranial and neral in *O.* × *citriodorum*, and linalool, methyl chavicol, eugenol, methyl eugenol and geraniol in *O. basilicum*. Based on a combination of the latter compounds, five major essential oil profiles could be distinguished in the accessions studied for *O. basilicum*. These profiles were largely the same for fresh and freeze-dried material of the same plant, although in dried leaves, methyl chavicol and eugenol concentrations had generally declined in comparison to those of linalool. There appeared to be little correlation between essential oil patterns and varietal classification within *O. basilicum*. In view of the chemical heterogeneity of *O. basilicum* and its use as an essential oil-producing crop, culinary herb, medicinal plant and insect-controlling agent, in all of which chemicals play an important role, the infraspecific classification of this taxon should take chemical characters into consideration. A system for the classification of essential oil chemotypes in *O. basilicum* is proposed. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The genus *Ocimum* L. (Lamiaceae) comprises *ca* 30 species which are found in tropical and subtropical regions [1]. They are rich in essential oils and have been the subject of numerous chemical studies [2, 3]. Many species have been grown by local people as medicinal plants, culinary herbs and insect-controlling agents, e.g. *O. americanum* L. (= *O. canum* Sims), *O. gratissimum* L., *O. tenuiflorum* L. (= *O. sanctum* L.) and *O. basilicum* L. The last plant, sweet basil, is also a major essential oil crop, estimated to produce annually 42.5 tonnes of oil worldwide [4]. Plants of *O. basilicum* typically have an aniseed-like aroma and sweet taste; the essential oil responsible for these features is methyl chavicol (=estragole) [5, 6]. However, wild populations differ in essential oil composition, and over the years many different chemocultivars varying in their aroma have been selected or bred by crossing with other cultivars or closely related species. The relationship among different forms of *O. basilicum* is, therefore, reticulate and the taxonomy of the group difficult. Paton and Putievsky [7] suggest that a system of standardized descriptors is needed to allow accurate identification and unambiguous communication of infraspecific taxa within the *O. basilicum* group, because morphological characters alone appear to be insufficient. By means of crossing experiments and chromosome counts they provide some of these descriptors. Pushpangadan and Bradu [8] gave a list of essential oil profiles characteristic of varieties of *O. basilicum*, suggesting that chemistry could provide additional characters to describe infraspecific taxa. The aim of the present study was to investigate whether this is the case and how much effect drying of the plant material has on the essential oil composition. For this purpose, the essential oil profiles were determined for both fresh and freeze-dried leaves of 16 different accessions of *O. basilicum* belonging to a number of different varieties and cultivars. Additionally, one accession of *O.* × *citriodorum* Vis. was investigated, which is thought to be a hybrid between *O. basilicum* and *O. americanum*. Analysis of extracts took place by means of GC–mass spectrometry.

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Table 1. Essential oil composition (%) of fresh leaves of *Ocimum basilicum* cultivars

	O. basilicum																
	Var. *basilicum* (flat leaves)						Var. *purpurascens*			Inter-mediate*	Cv. Dark Opal		Var. *basilicum* (convex leaves)			Var. *difforme*	*O.* × *citrio-dorum*
Essential oil	17	122	130W	145	147/97	SW	74	76	147/13	130P	100Y	100R	R-1	R-3	US	203	197
Sabinene													1.2	0.2	0.3	0.7	
Myrcene								1.1	1.0	0.9		0.8	1.1	0.7	0.8	2.2	0.4
1,8-Cineole (+limonene)	6.1	2.2	3.8	4.1	9.4	6.8	2.4	11.4	3.7	6.8	9.0	6.1	14.7	6.6	9.3	19.9	0.7
Ocimene							2.3	1.0	3.5				1.0	0.7	0.9		
Fenchone					2.2						2.6	1.4					
Linalool	30.6	79.4	85.6			40.9		32.8	15.7	69.3	38.6	30.5	31.8	29.9	26.7	15.6	1.9
Camphor							1.5		2.5			2.7	0.7		0.6	2.1	
4-Terpineol																	2.3
Methyl chavicol	53.4	0.5		91.5	45.3		89.8	0.6	56.5				43.7	56.1	55.6	45.5	
Neral																	39.8
Geraniol									8.5								
Geranial									1.7								50.9
Eugenol		4.6				41.3	1.1	39.6			38.9	48.0					
β-Elemene	1.0	2.3	1.2			3.7		5.2	2.2	1.2	6.2	1.6	1.5	1.2	0.6	1.2	
Methyl eugenol					43.2							5.8					
β-Caryophyllene				4.4													3.2
Bergamotene	2.0	5.1	3.0			3.5	0.7	1.1	1.1	12.7					0.8	4.9	
α-Humulene													0.7	0.6			0.4
β-Bisabolene															1.1	1.5	0.5
γ-Cadinene	2.9	3.0	3.2			1.6	0.9	3.9	1.5	4.7	2.0	1.0	1.6	1.5	1.2	2.4	
τ-Cadinol	3.9	3.0	3.2			2.2	1.3	3.3	2.2	4.3	2.7	2.3	2.1	2.5	2.1	3.9	

*Intermediate between var. *basilicum* and var. *purpurascens*.

Table 2. Essential oil composition (%) of dried leaves of *Ocimum basilicum* cultivars

	O. basilicum																*O.* × *citriodorum*
	Var. *basilicum* (flat leaves)						Var. *purpurascens*			Inter-mediate*	Cv. Dark Opal		Var. *basilicum* (convex leaves)			Var. *difforme*	
Essential oil	17	122	130W	145	147/97	SW	74	76	147/13	130P	100Y	100R	R-1	R-3	US	203	197
Sabinene																	
Myrcene		3.0	1.6	0.8				6.8	2.2	1.0	2.1	3.4	2.0	0.6	0.6	4.5	2.6
1,8-Cineole (+limonene)	3.1	2.3	2.6	4.2	2.2	1.4	0.4	11.8	4.7	1.5	6.6	6.6	9.9	4.0	6.2	13.8	2.4
Ocimene							0.2	4.1	1.1		2.3	2.9	1.4	0.3			
Fenchone				0.6	0.5						1.4	2.2					
Linalool	46.7	78.1	84.4	0.6		47.3	0.5	50.0	25.9	85.4	36.0	42.3	49.6	56.7	48.6	24.8	4.5
Camphor							1.2		2.4			3.6	2.3	0.6		3.1	
4-Terpineol																	5.2
Methyl chavicol	29.0	2.4	0.5	83.8	39.8	0.8	88.3		18.6				19.8	24.2	37.3	34.5	
Neral																	26.3
Geraniol									23.0	1.4							
Geranial			1.8						3.4	0.8							51.4
Eugenol		2.5		0.3	0.7	28.3		18.9	1.3	0.5	38.5	25.1			0.7		
β-Elemene	2.4	1.6	1.9			4.1	2.5	2.2	3.3	3.0	2.9	2.8	4.8	2.9	0.7	1.7	
Methyl eugenol				0.7	43.8		1.0		0.4		1.1	2.0					
β-Caryophyllene				6.1	10.4						2.4	2.0					4.7
Bergamotene	2.0	2.2	1.4			1.4	1.0		2.1	1.3						2.7	
α-Humulene				1.0									2.3	1.9	0.4		1.3
β-Bisabolene	1.9		1.7	0.6		3.3										4.1	1.6
γ-Cadinene	5.5	2.2	1.6			4.1	1.7	2.7	3.8	1.5	1.8	1.5	2.2	2.9	1.4	3.6	
τ-Cadinol	8.6	3.9	2.4	0.1	0.5	0.7	2.6	3.5	5.5	2.5	3.2	2.8	3.3	4.4	3.2	4.6	

*Intermediate between var. *basilicum* and var. *purpurscens*.

RESULTS AND DISCUSSION

Thirty different essential oils, including all the major constituents, were identified in the GC analyses of the various *O. basilicum* extracts by means of their retention times and mass spectral fragmentation patterns. Additional minor components (mainly sesquiterpenes) were present in some accessions, but could not be identified with certainty. In Tables 1 and 2 the occurrence of the major and most frequently encountered minor components are listed (in sequence of their retention times), together with their percentages of the total essential oil content in the different accessions; Table 1 gives the results for the fresh leaves and Table 2 for the dried plant material. The essential oil compositions appeared to be very different in the different accessions (=genotypes, see Experimental). The major essential oil components, i.e. those which comprised 20% or more of the total in at least one genotype, were especially variable in occurrence and concentration among the different accessions, ranging from absent in some genotypes to more than 90% of the total essential oil composition in others. Those major constituents were: linalool, methyl chavicol, eugenol, methyl eugenol, geraniol, geranial and neral. The structures of these compounds are given in Fig. 1, apart from neral, which is the *cis*-isomer of geranial. Figure 2 shows the contributions of each of the seven main components to the total essential oil composition for each of the 17 accessions studied. There appear to be five basic essential oil profiles in the *O. basilicum* plants investigated: (a) linalool as the major compound (accessions NY B 122, 130W and 130P); (b) methyl chavicol as the major component (NY B 145 and 74); (c) a mixture of linalool and methyl chavicol as the two major constituents (NY B 17, R-1, R-3, US and 203); (d) a mixture of linalool and eugenol (NY B SW, 76, 100Y and 100R); and (e) a mixture of methyl chavicol and methyl eugenol (NY B 147/97). The profile of NY B 147/13 is a variation of (c) with geraniol as a third major component. The one accession studied for *O.* × *citriodorum* (NY B 197) showed a totally different essential oil composition pattern (f), consisting of geranial and neral (=*trans*- and *cis*-citral, respectively) as the principal compounds.

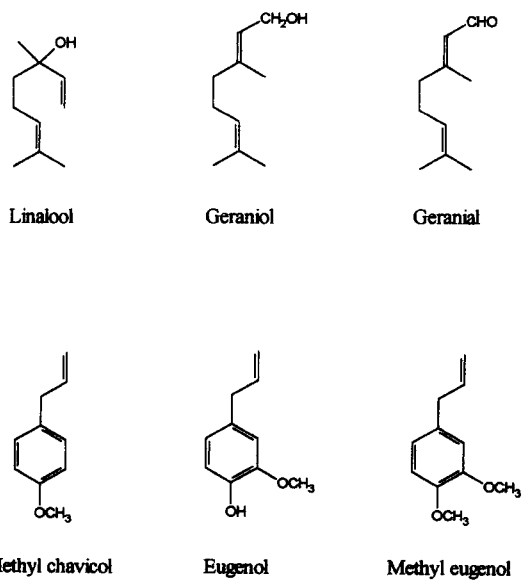

Fig. 1. Structures of major essential oils found in *Ocimum basilicum* and *O.* × *citriodorum*.

A comparison between the essential oil compositions of fresh and dried leaves (see Tables 1 and 2 and Fig. 2) reveals that many small and some larger changes have taken place during freeze-drying and/or storage of the plant material for six months. In particular, the ratios of methyl chavicol to linalool and of eugenol to linalool appear to have decreased. This means that the phenylpropanoids methyl chavicol and eugenol must have deteriorated or evaporated much more rapidly than the monoterpenoid linalool. Likewise, the monoterpenoids geraniol and geranial also appear to have been preserved better on drying than methyl chavicol (compare data for fresh and dried leaves of accession 147/13). The relatively rapid decrease of methyl chavicol and eugenol in dried basil leaves has been reported before [9]. Despite this deterioration, the overall essential oil patterns (a)–(e) recognized by us in *O. basilicum* and the profile of *O.* × *citriodorum* were still easily recognizable in plants stored for six months, so that these profiles indeed appear to be potential descriptors for the genotypes of these plants, no matter whether fresh or dried leaves are used for study.

On the basis of more than 200 analyses of oils in *O. basilicum*, Lawrence [4] recognized four major essential oil chemotypes in this species, each with a number of small variants: (1) methyl chavicol-rich; (2) linalool-rich; (3) methyl eugenol-rich; and (4) methyl cinnamate-rich. No eugenol-rich plants were found during these studies. In other reviews, e.g. by Pushpangadan and Bradu [8], chemotypes are classified according to the percentages of all the major constituents, which apart from the four major essential oils mentioned above also include eugenol. According to Lawrence's chemotype classification [4], cultivars containing two or more major essential oils could have been classified differently before and after drying. For instance, fresh material of accessions NY B 17, 76 and 147/97 would have been considered as belonging to the methyl chavicol-rich, eugenol-rich and methyl chavicol-rich groups, respectively, but dried leaves of the same accessions would have been classified as linalool-rich, linalool-rich and methyl eugenol-rich, respectively, because the second most common constituent has now become the major one. Therefore, there is clearly a problem using essential oil chemotypes based on just one major volatile, because frequently there are two or more major compounds which may be present in nearly equal amounts, and it probably would be better to assign essential oil profile classes based on all the major constituents (e.g. constituting more than 20% of the total essential oil content), even if there are three or four major volatiles. Moreover, not too much weight

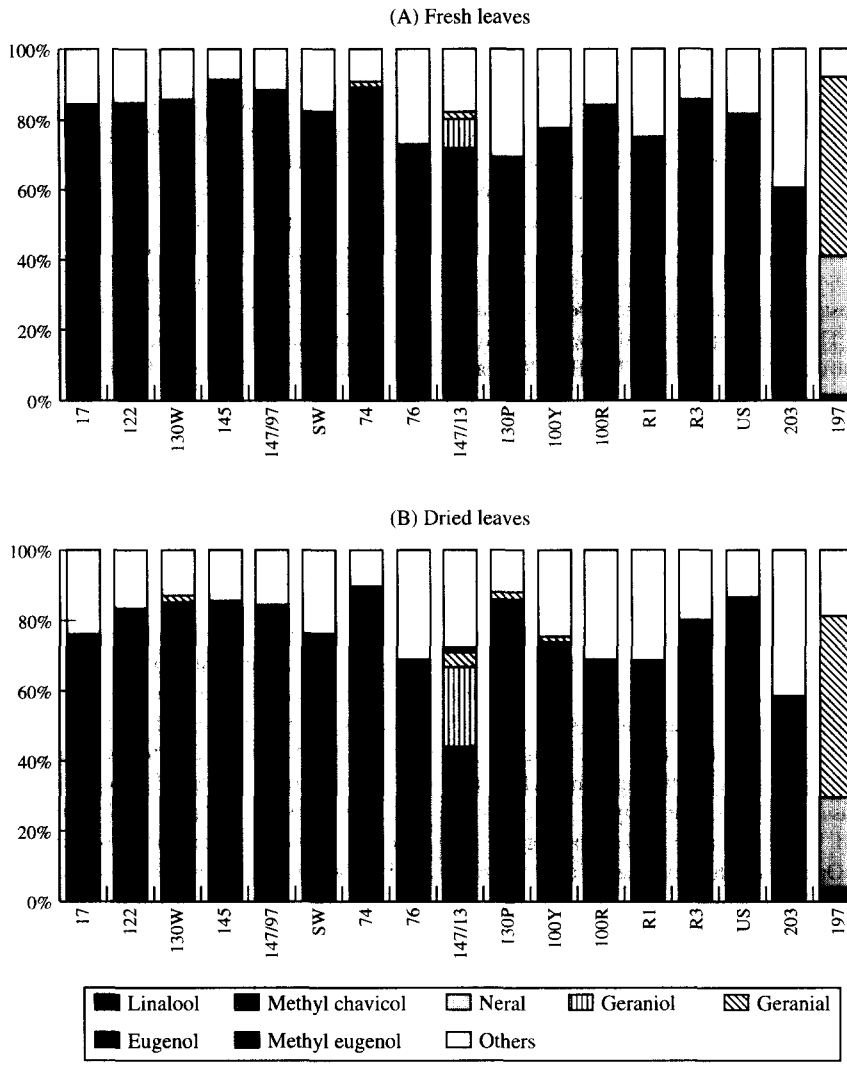

Fig. 2. Major essential oil profiles found in fresh and dried leaves of genotypes of *Ocimum basilicum* and *O.* × *citriodorum*.

should be given to the exact percentages in which these major compounds occur, because they are likely to change after drying. Apart from using fresh or dried plant material there are other factors which may potentially affect the essential oil percentages determined, such as the extraction method employed [10], the age and organ of the plant used for study [11–13] and the environmental conditions under which the plants have been grown. For instance, essential oil compositions may be slightly different in the same genotype depending on whether the plants have been grown in a greenhouse or outside [14, 15], whether fertilizers have been applied or not [16] and depending on the light regime [17]. Therefore, for comparative studies, plants should be grown under as identical conditions as possible. Different methods of extraction did not seem to have much effect on the major essential oil profiles we determined. Because the amount of plant material available for chemotaxonomic studies is usually small, we used solvents for the extraction of essential oils rather than steam distillation. Diethyl ether was preferred as an extractant for the dried material and dichloromethane for fresh leaves, the first solvent being less harmful than the latter, but for fresh plants diethyl ether may be less efficient an extractant than dichloromethane in the presence of some water (dew or condensation on the fresh leaves). In a separate experiment (Putievsky, unpublished results), the essential oil compositions of the same genotypes were also determined after steam distillation, and the results for most genotypes were similar to those determined after solvent extraction

The plant accessions used for the present study were all of the same age (advanced flowering stage), they were grown under the same conditions, and the same organs (leaves) were extracted, so that the results are comparable and differences in chemical profiles should reflect genetical differences between the various accessions. Figure 2 shows that all five major essential oil profiles (a)–(e) were found in the six accessions investigated for *O. basilicum* var. *basilicum* with flat leaves (NY B 17, 122, 130W, 145, 147/97 and SW), and that

three different patterns [(b), (d) and a variation of (c)] were present in the three accessions studied for var. *purpurascens* (NY B 74, 76, 147/13). Therefore, these varieties, which are based on morphological features, are not characterized chemically. More correlation between morphology and chemistry was found in the convex-leaved forms of var. *basilicum*; the three accessions studied (NY B R-1, R-3 and US) belonged to the same essential oil chemotype (c). This together with the fact that they have the same chromosome numbers (see Table 3) suggests that these taxa may be genetically closely related. The same applies for the two accessions studied for cultivar 'Dark Opal' (NY B 100R and 100Y), which are also very similar in their essential oil [chemotype (d)].

Comparison of our results with the data presented by Pushpangadan and Bradu [8] for the different varieties of *O. basilicum* reveals both similarities and differences. They found three chemotypes in their var. *glabratum* Benth. (which is included in our var. *basilicum*), including the linalool/methyl chavicol profile which we found in NY B 17, R-1, R-3 and US, and the linalool/eugenol profile found in NY B SW. However, none of our cultivars corresponded to their third chemotype, linalool/eugenol/camphor (40, 20 and 20%, respectively). The pattern they described [8] for var. *purpurascens* (linalool, 60%; and methyl cinnamate, 20%) was also absent from any of the cultivars we investigated for this variety. The chemotype for cultivar 'Dark Opal' (which they treated as a variety) showed some similarity with our results; they reported linalool (35%)/geraniol (35%)/eugenol (20%), whereas we encountered linalool/eugenol (NY B 100R and 100Y). Plants of Pushpangadan and Bradu's var. *crispum* (Thunb.) E. A. Camus (= var. *difforme* Benth.) showed the same chemotype as that of the one accession we studied (NY B 203): linalool/methyl chavicol (the latter compound in higher concentrations than the former). Finally, they found citral (=geranial + neral) to be the major constituent in *O.* × *citriodorum*, which agrees with our results (see accession NY B 197). The general conclusion is that some of the varieties of *O. basilicum* are rather heterogenous as far as their major essential oil profiles are concerned, e.g. var. *basilicum* and var. *purpurascens*, whereas the members in other infraspecific taxa seem to be more similar to each other in this respect. On the other hand, apparently unrelated taxa may show the same essential oil profile. For instance, NY B 100R and 100Y share their chemotype (d) with the 'unrelated' genotypes NY B SW and 76, which belong to two different *O. basilicum* varieties, and NY B R-1, R-3 and US share their essential oil profile (e) with NY B 17 and 203 which also belong to different varieties. Thus, varieties based on morphology do not necessarily match chemotypes and vice versa, and this suggests that morphological and chemical characters segregate during breeding. These complicated and reticulate relationships are not restricted to *O. basilicum*; in fact, infraspecific chemical variation is found in many essential oil plants in the Lamiaceae, such as species belonging to the genera *Mentha* and *Thymus* [18]. Indeed, this phenomenon is not restricted to Lamiaceae and essential oil characters. For instance, in the medicinal plant *Gratiola officinalis* L. (Scrophulariaceae) there are two totally different flavonoid glycoside profiles which are not correlated with differences in morphological characters [19].

In many cases, infraspecific and even infravarietal chemical variation does not pose much difficulty for the taxonomy of plants; since morphological characters are the most obvious ones, and perhaps easiest to study, classifications should in the first instance be based on

Table 3. Details of *Ocimum* genotypes used for essential oil studies

Species and variety	Accession	Origin	Chromosome no. (2*n*)*
O. basilicum var. *basilicum*			
(flat leaves)	NY B 17	Italy	56
	NY B 122	Holland	56
	NY B 130W	India	52
	NY B 145	Yemen	72
	NY B 147/49	U.S.A.	74
	NY B SW	Brazil	52
O. basilicum var. *basilicum*			
(convex leaves)	NY B R-1	U.S.A.	52
	NY B R-3	U.S.A.	52
	NY B US	U.S.A.	52
O. basilicum var. *purpurascens*	NY B 74	Thailand	52
	NY B 76	Israel	52
	NY B 147/13	Unknown	53
Var. *basilicum/purpurascens*			
intermediate	NY B 130P	India	52
O. basilicum var. *difforme*	NY B 203	U.K.	56
O. basilicum cv. Dark Opal	NY B 100R	U.K.	52
	NY B 100Y	U.K.	52
O. × *citriodorum*	NY B 197	Thailand	64

*See ref. [7].

morphology. However, in essential oil-containing plants the chemicals are sometimes more important characters than morphological features. This applies not only to the economic uses of the plant, such as essential oil extraction for the chemical industry, but also for research purposes. After all, medicinal properties and biological activity of a plant are usually due to its chemical profile. Therefore, reports on the pharmacological or pesticidal activity of crude extracts of plants such as *O. basilicum* have little value if the chemotype has not been determined, and chemical characters are absolutely essential additional descriptors for the infraspecific classification, since morphological features alone are inadequate to describe the many different facets of the taxon under study. There is currently no agreed system of chemotype classification. We propose that the system of chemical descriptors should be based on the combination of major chemical components rather than the sole dominant compound.

EXPERIMENTAL

Plant material. 16 Accessions belonging to 3 varieties and 1 cultivar of *O. basilicum* and 1 accession of *O.* × *citriodorum* were raised from seed in the winter of 1994/1995, and grown in pots in a heated greenhouse at the Royal Botanic Gardens, Kew. The variety, code, origin and chromosome number of each accession are given in Table 3. Voucher specimens have been deposited at the Kew Herbarium. Each accession was considered to be a genetically homogeneous line (genotype). In the summer of 1995, the plants were put outside to receive natural sunlight for 1 month before they were collected for chemical analysis or freeze-drying.

Extraction of fresh leaves. Fresh leaf material (*ca* 5.0 g) for each cv. taken from the same position of the plant, was rinsed for 20 sec in a vial containing 10 ml CH_2Cl_2. An aliquot of this extract was concd 10× under N_2 for analysis by GC-MS.

Extraction of dried leaves. Freeze-dried leaf material (500 mg) was ground with a pestle and mortar using acid-washed sand and a small vol. of Et_2O. The ground material and Et_2O were transferred to a vial, 5.0 ml Et_2O was added, the vial was closed, and the leaf material was left to extract for 1 hr. An aliquot was concd 10× under N_2 for GC-MS analysis.

GC-MS analysis. Essential oil extract (1 μl) was analysed by GC-MS using a Perkin-Elmer Model 8500 GC coupled to a Finnigan-MAT Ion Trap MS. GC conditions were: column, 25 m × 0.22 mm i.d. × 25 μm BPX5 (SGE); oven programme, 60° (1 min) 60–200° (4° min^{-1}) 200–340° (8° min^{-1}) 340° (10 min); carrier gas, He at 20 psi; injection, split or splitless at 350°. MS conditions were: ionization, EI at 70 eV; m/z range, 40–400; scan rate, 1 sec^{-1}. Identification of sepd components in the extract was achieved by comparison with published RR_ts and mass spectra [20] or with purchased standards. The eluent from the GC column was also split to a FID to allow simultaneous acquisition of percentage composition data.

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