

Phytoplenolin[®] *

A Natural Product

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*Phytoplenolin (R) as used in this monograph is the same as Plantolin-Australia (R), registered by Koori marketing Pty, Ltd, and Bio-Botanica Inc

Abstract

Phytoplenolin (R) is a trade name registered by Bio-Botanica, Inc. for *Centipeda cunninghami* A. Br. & Aschers. and the unique extracts prepared thereof. *C. cunninghami* has been used since ancient times in folk-medicine in Australia and the Far East mainly for different allergic and common cold manifestations. A standard ethanolic extract containing 0.2% sesquiterpene lactones was tested for topical antiinflammatory properties when it compared with a standard antiinflammatory preparation containing 1% hyaluronic acid in water, it also inhibited alpha-2-phospholipase enzyme at comparatively low concentrations. The same extract compared favourably with a standard cell renewal preparation containing 2% glycolic acid using Dansyl chloride cell renewal test. A distinct sunscreen activity was also shown. The essential oil of the plant was investigated by GC/MS leading to the identification of 44 compounds constituting 90% of the oil. The major constituents are cis-dihydrocarveol (23.98%), trans-sabinyl acetate (22.97%) and trans-chrysanthenyl acetate (13.50%). Myrtenol constituted 5.85%; thymol, 1.5%; isolamyl caproate, 1.23% and isobutyl isopentanoic acid ester, 1.18%.

The special extraction procedure and the mentioned biological activities of the extract are presently U.S.A. Patent Pending filed by the authors.

Introduction

Centipeda cunninghami A. Br. & Aschers is commonly known as "Sneezeweed" or "Old Man's Weed". It is native to Australia where it has been used by aborigines for burns, wounds, skin infections,

diarrhea and rheumatism (1). In Chinese traditional medicine the plant has been used to treat colds, nasal allergies, asthma, Malaria and amoebiasis (2). A plant containing paint for room application for health promotion was even prepared and patented (3). Antiallergic (4), antiprotozoal (2) and platelet activating factor antagonistic (5) activities were attributed to the sesquiterpenoid constituents which may be found in the essential oil. Even antimutagenic (6) and antiviral (7) activities were attributed to the plant. In a previous study (8) (-)-cis-Chrysanthenyl acetate has been isolated by fractional distillation of the volatile oil, however, according to the available literature, no systematic investigation has been carried out on the essential oil which is responsible for certain activities of *C. cunninghami* A. Br. & Aschers. Due to the important systemic effects of the plant it was also deemed interesting to explore the effectiveness of the plant when applied topically.

Results and discussion

Leaves and flowering tops of *C. cunninghami* A. Br. & Aschers., were brought to the laboratory. The volatile oil was steam distilled from the carefully dried plant material, giving a cloudy distillate with very little oil separating at the surface indicating that the specific gravity of the oil was slightly lower than that of water ($d_{20} = 0.995$), therefore, the oil was extracted from the distillate with diethyl ether yielding 0.1% of an amber-yellow volatile oil with penetrating characteristic aromatic odor, soluble in light petroleum, benzene, chloroform, ether and absolute ethanol. Refractive index ($n_{20} = 1.473$).

Analysis of the oil was carried out by GC/MS under condition that allowed direct comparison with reported data on reference terpenes (9). Both retention times and mass spectral fragmentation were used in the identification of the individual



Centipeda cunninghami - Close up fig 1.

components of the oil. Table 1 shows a list of all components and their relative percentage in the oil. For all unidentified components the most significant mass spectral fragments and retention times (in seconds) are provided.

The composition is determined based on relative



Centipeda cunninghami
Illustration



Centipeda cunninghami - Close up fig 2.

response in the chromatograms. The identification of each component is based on direct comparison of the retention time and mass spectral fragmentation with the data published by Adams (9) and others (10-13). The mass spectral library fit (Wiley 139 Library) for all identified components was more than 90%. It should be mentioned that the GC column used in this study (Rtx®-5, 30m x 0.25 mm, 0.25um film thickness) was similar to that reported by Adams (9).

The data presented in table 1 showed that the oil is composed of at least sixty-two distinct peaks. Forty-four compounds have been unequivocally identified constituting more than 90% of the oil. *cis*-Dihydrocarveol (23.89%) *trans*-sabinyl acetate (22.97%) and *trans*-Chrysanthenyl acetate (13.50%) were the major constituents; myrtenol constituted 5.85%; thymol, 1.5%; isoamyl caproate, 1.23%; and isobutyl isopentanoic acid ester, 1.18% (Table I).

Phytolenol(R) belongs to the Daisy Family (Asteraceae, Compositae). it is an aromatic perennial herb about 30cm high, with quite woolly young shoots. Stem, green, cylindrical, longitudinally striated with short internodes; leaves (1-3cm) are green, simple sessile, alternate, oblong-ovate, margin, dentate, apex acute, venation, pinnate reticulate, midrib conspicuous, prominent on the lower side.



Centipeda cunninghami In field - Australia - fig 3.

Table 1. The chemical composition of the essential oil of *Centipeda cunninghami*

<u>a No.</u>	<u>Components</u>	<u>%</u>	<u>a No.</u>	<u>Components</u>	<u>%</u>
1	Methylbutyl acetate	0.28	34	Unknown Rt=25.350, MS:M ⁺	
2	n-Amyl acetate	0.75		136, 94, 59*, 43	0.41
3	alpha-Pinene	0.78	35	Myrtenol	5.85
4	beta-Pinene	0.22	36	Cis-Cerveol	0.18
5	trans-Geraniol	0.12	37	3, 3-Dimethyl hexanol	0.17
6	Unknown: R _i =15.499, MS:M ⁺		38	Unknown Rt=26.091, MS:M ⁺	
	134,103, 92, 91* 85, 65, 57 43	.013		154, 107, 99, 77, 71, 70*	0.94
7	Isobutyl isovalerate	0.68	39	Isoamyl caproate	1.23
8	Unknown Rt=16.014, MS:M ⁺		40	Anisole, O-isopropenyl	0.82
	136, 115, 89, 71, 70, 55, 43*	0.37	41	trans-Crysanthenyl acetate	13.50
9	Isobutyl isopentanoic acid ester	1.18	42	Acetanisole	0.40
10	Unknown Rt=16.435, MS:M ⁺		43	Ascaridole	0.14
	134, 119*, 117, 91, 77, 65, 57, 41	0.19	44	Unknown Rt=30.102, MS:M ⁺	
11	beta-Phellandrene	0.10		150, 135*, 115, 91, 77, 51, 40	0.13
12	gamma-Terpinene	0.10	45	Thymol	4.64
13	Benzyl ethyl carbinol	0.18	46	Geranyl acetate, 2, 3-epoxy	0.10
14	trans-Carveol	0.17	47	Hexyl caproate	0.34
15	Amyl valerate	0.33	48	trans-Sabinyl acetate	22.97
16	2-Methylbutyl 2-Methylbutyrate	0.64	49	Unknown Rt=32.998, MS:M ⁺	
17	3-Methylbutyl ester (Apple Oil)	0.67		162, 148, 134, 119, 105, 91, 98, 65, 57*, 44	0.09
18	Pentyl 3-methyl butanoate	2.09	50	alpha-ylangene	0.29
19	Unknown Rt=21.117, MS:M ⁺		51	Unknown Rt=34.784, MS:M ⁺	
	148, 117, 99, 87, 71, 55, 56*, 43	0.16		164, 148, 135, 108, 105, 104*, 91, 65, 57	0.13
20	Unknown Rt=21.455, MS:M ⁺		52	Unknown Rt=34.893, MS:M ⁺	
	154, 89, 84, 71, 69, 56, 43*	0.33		204, 119, 104, 91, 71, 43*	0.64
21	cis-Sabinene hydrate	0.27	53	Unknown Rt=35.214, MS:M ⁺	
22	trans-3(10)-Caren-4-ol	0.92		160*, 159, 131, 103, 77, 51, 43	0.10
23	trans-(-)-Pinocarveol	0.36	54	trans-Caryophyllene	0.17
24	Trans-Sabinene hydrate	0.13	55	Myrtenyllacetate	0.23
25	Unknown Rt=22.867, MS:M ⁺		56	Allylanisole	1.5
	134, 117, 106, 96, 80, 77, 68*57, 40	0.21	57	Unknown Rt=38.701, MS:M ⁺	
26	Unknown Rt=22.959, MS:M ⁺			150, 135, 115, 91, 77, 71, 43*	3.5
	154, 119, 109, 94, 79, 65, 59*,43	0.33	58	beta-Phenylethyl isovalerate	0.72
27	p-Anisaldehyde	0.13	59	Unknown Rt=39.404, MS:M ⁺	
28	Myrtenal	0.17		254, 147, 117, 91, 77, 73, *, 57, 40	0.09
29	cis-Dihydrocarveol	23.89	60	Unknown Rt=42.455, MS:M ⁺	
30	p-Mentha-1, 5-Dien-8-ol	0.69		234, 150, 135, 105, 85, 57*, 41	1.22
31	Terpinene-4-ol	0.94	61	Nerol	0.53
32	p-Cymene-8-ol	0.58	62	Unknown Rt=43.379, MS:M ⁺	
33	(-)-cis-Caran-trans-3-ol	0.21		136, 121, 104*, 71, 51, 43	0.08

*a Components are numbered in order according to their Rt, *Base Peak*

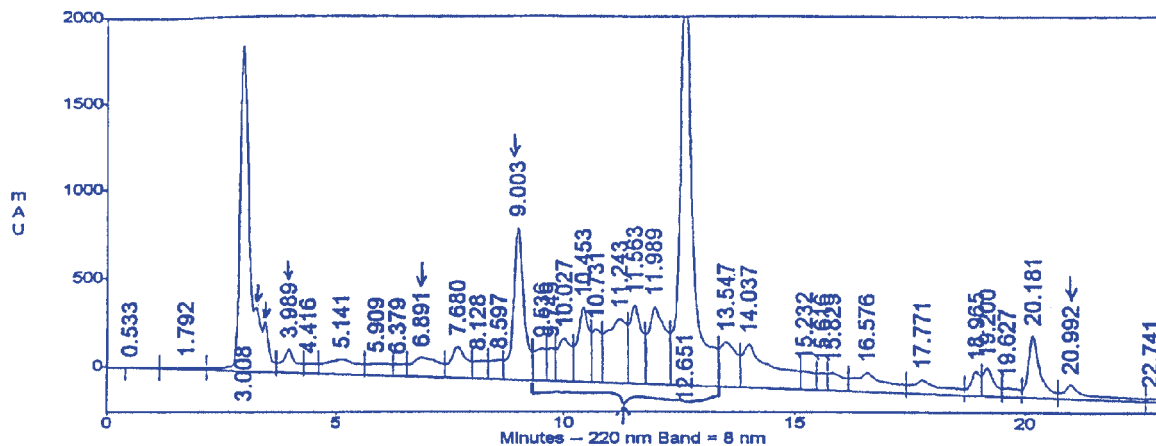


Fig 4

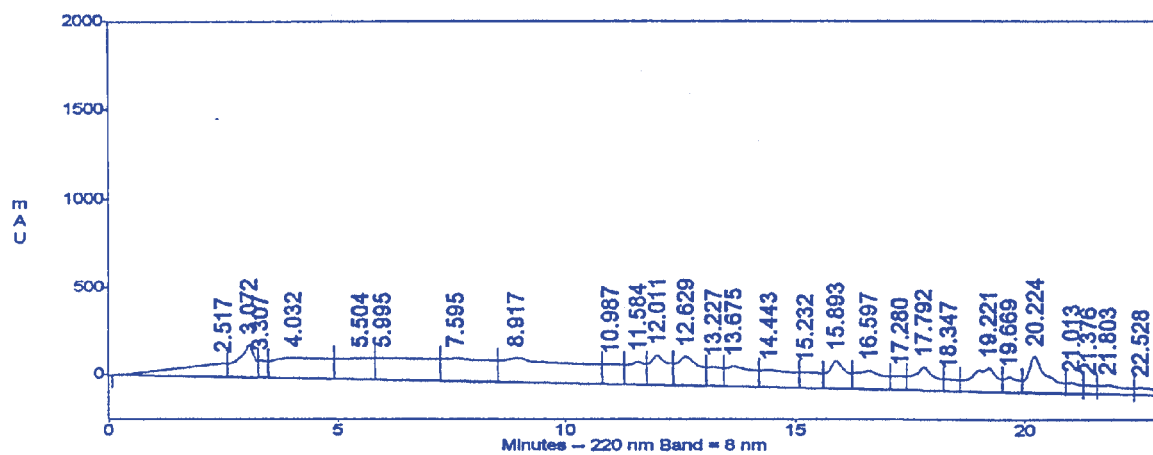


Fig 5

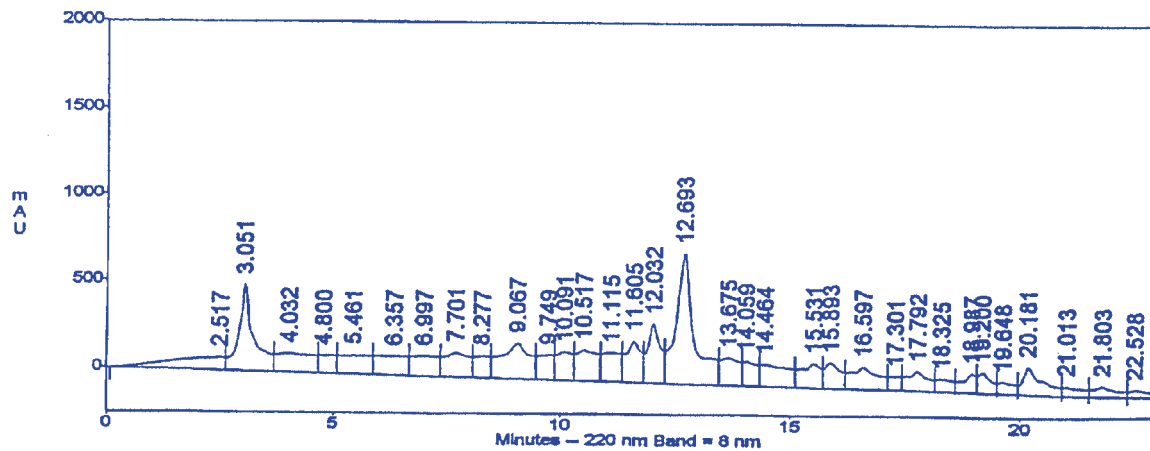


Fig 6

Odor, characteristic, aromatic, sternutatory; taste, aromatic, slightly bitter. The flower heads are small (0.5-0.8cm in diam.) greenish and sessile. (Fig. 1, 2 and 3)

A unique holistically balanced extract was prepared by a special procedure developed by Bio-Botanica. A patent has been filed with the method of preparation of the special extract done by Bio-

Botanica, Inc. and the biological activities of this extract.

Fig. 4 shows the HPLC chromatogram of Bio-Botanica's product as compared to the products mentioned in the literature and prepared by either 95% ethanol (Fig. 5) or 50% ethanol (Fig. 6). Fig. 7 is a three dimensional HPLC chromatogram showing the most important sesquiterpene lactones peaks.

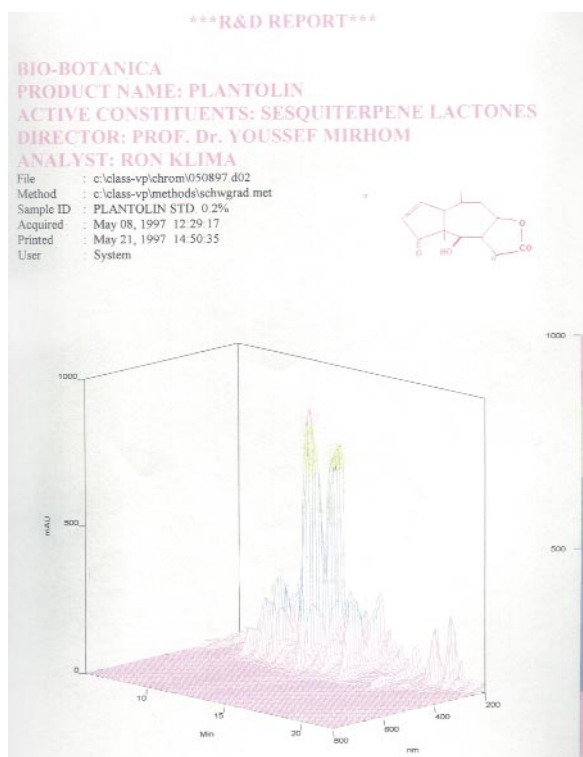


Fig 7.

Before exploring the effectiveness of Bio-Botanica's extract when used topically it was deemed important to test in addition its safety on human skin. Therefore, the appropriate biological studies were performed by an independent laboratory*. The results were as follows:

1) The test used to prove the safety of Phytolenolin(R) the Hen's Egg Test utilizing the chorioallantoic Membrane (HET-CAM) which proved to be more sensitive to liquid irritants than is the rabbit eye. Phytolenolin(R) at 10% concentration had practically no ocular irritation potential.

2) To test the anti-inflammatory activity two experiments were carried out:

a) In the first experiment, the extract potential to decrease a chemically (lactic acid) induced erythematous response was evaluated. The test was performed on five carefully selected human subject and the percentage reduction in erythema (anti-inflammatory activity) was measured, when Phytolenolin (R) compared almost equally with a standard 1% hyaluronic acid solution which is a well known moisturizing, soothing and emollient agent. Phytolenolin(R) at 0.2% concentration alleviated inflammation as much as a 1.0% hyaluronic acid (5 times higher concentration) which is highly expensive.

b) In the second anti-inflammatory experiment the potential of the extract to inhibit

alpha-2-phospholipase enzyme was evaluated since it is known that activation of this enzyme causes inflammation. In this case an extract at a concentration of 0.025% sesquiterpene lactones showed to be as active as a preparation of Caribbean Gorgonians of the genus Pseudopterogorgia containing 0.06% pseudopterosins which possess a well proven and patented anti-inflammatory effect. (14)

3) In this clinical experiment we tried to explore the possible healing effect of the extract. Six human subjects were carefully selected in order to evaluate Dansyl chloride cell renewal properties.

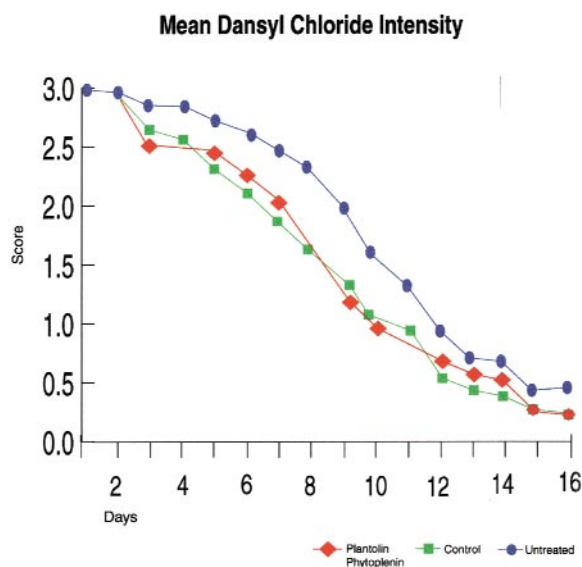


Fig 8.

Table 2. Mean Dansyl Chloride Intensity Scores

Days	Phytolenolin	Control	Untreated
1	3.00	3.00	3.00
2	3.00	3.00	3.00
3	2.58	2.67	2.83
4	2.58	2.58	2.83
5	2.33	2.25	2.67
6	2.17	2.08	2.58
7	1.92	1.83	2.42
8	1.67	1.67	2.25
9	1.42	1.50	2.00
10	1.08	1.00	1.50
11	0.67	0.83	1.33
12	0.50	0.42	0.92
13	0.50	0.42	0.75
14	0.42	0.33	0.75
15	0.25	0.25	0.58
16	0.17	0.17	0.58

In this test a dye [Dansyl chloride, (5-methyl-amino-1-naphthalene-sulphonyl chloride)] is applied to the skin leaving a dye stain which disappears proportionally with the characteristic power of the applied preparation to induce the formation of new cells which displace the dye colored cells. The preparation (0.2% sesquiterpene lactones) was compared to a standard 2% alpha-hydroxy-acid lotion (known to be a potent cell renewal agent). Phytolenin (R) gave a 50% reduction in the stain as compared to the untreated site of the skin after 11 days while the standard preparation gave only 38% reduction after the same period of time (see Table II & Fig. 8) this means that the 50% cell renewal response was attained more quickly by Plantolin(R) which was applied at a much lower concentration of 0.2% (alpha-hydroxy-acid preparation, 2.0%).

In other words Phytolenin (R) is very effective as a cell renewal agent at comparatively very low concentration without the drawbacks of the drastic, mechanical, exfoliating and caustic effect of the alpha-hydroxy-acids. On the contrary the extract is anti-inflammatory as proven above while acting as a smooth cell renewing agent. It is, therefore, undoubtful - in our opinion- that consumers will turn to the use of Phytolenin for cell renewal in the near future.

4) The efficacy of Phytolenin (R) as a sunscreen product was tested on five carefully selected human subjects. The Sun Protection Factor (SPF) was determined taking a preparation of 8% Homosalate (SPF=4.0) as standard. Phytolenin (R) preparation gave an average $SPF \pm SE = 1.68 \pm 0.08$ viz even if the SPF of Phytolenin (R) is not too high it should protect against sunburn due to its potent anti-inflammatory property and will be very helpful if added to sunscreen products.

In conclusion it is too good to believe that we have at hand a product with potent Anti-inflammatory, cell renewal (healing) agent with distinct sunscreen activity. If these effects, which were demonstrated by biological and clinical studies carried out by a reputable laboratory, are combined to the effects cited in the literature as antihistaminic (4) antimutagenic (6) and antiviral (7) agent, it is easy to understand the effectiveness of Plantolin(R) preparations in Psoriasis and Herpes Simplex (15). Moreover, it will not be difficult to conceive that Phytolenin(R) will definitely cause a new Revolution in the cosmetic and Skin Care Industry.

Experimental

Plant Material:

The flowering tops together with the leaves of *Centipeda cunninghami*, A. Br. & Ascher., Fam. Asteraceae were collected early in the morning of December 7, 1995, from the District of Kinglake, Victoria, Australia, from special plots grown to Industry Standards over a period of nine years. The plant was dried at room temperature on racks in the shade. It was identified by R. W. Taylor, Horticulturist, Government Researcher and T. V. Wunnik ND,DC. Fellow A.N.T.A. to whom the authors are indebted. A Voucher sample is kept at the herbarium of Bio-Botanica, Inc. at Hauppauge, New York.

Preparation of the Volatile Oil, Quantitative Determination and Physical Constants:

100g of plant material were accurately weighed and steam distilled in a glass distillation apparatus for volatile oils lighter than water. The oil floating on the surface (which was minimal), together with the turbid distillate in the stem of the receiver were extracted with diethyl ether (3x25 ml) dried over anhydrous sodium sulphate and the ether evaporated spontaneously at about 28°C in a fuming cupboard. The oil obtained was measured and the percentage yield calculated. Refractive Index and Specific Gravity determinations were carried out at 20°C as described in U.S.P.XXIII.

Reference Standards

Reference standards of different monoterpenes, sesquiterpenes, and alkanes were obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI), Fluka Chemical Corporation, (New York, NY), Roth Co. Chemische Fabrik (Karlsruhe, Germany), Sigma Chemical Co. (St. Louis, MO) and Varian Associated (Houston, TX). These reference standards include: Tricyclene, alpha-pinene, sabinene, alpha-phellandrene, 1-decene, limonene, fenchone, alpha-terpineol, alpha-terpinolene, cis-verbenol, cis- and trans-carveol, cis-dihydrocarveol, alpha-longipinene, alpha-cedrene, (-)-isolongifolol, alpha-humulene, valencene, cuparene, myristyl alcohol, citronellyl acetate, neryl acetate, geranyl acetate, camphene, alloaromadendrene, n-icosane, and n-heneicosane. Solutions were prepared in methanol at concentrations of 10 mg/ml. For GC/MS analysis each standard solution was diluted by mixing 0.1 ml of the standard solution with 0.9 ml of methanol.

Volatile Oil Solution

For GC/MS analysis the volatile oil was dissolved in pentane (98%), b.p. 36°C at a concentration of 10% by volume.

GC/MS Analysis

A Shimadzu GC-17A with a capillary injector and interfaced to a QP 5000 Shimadzu Mass Spectrometer was used. The data were recorded using AST Bravo LC 5100 Pentium computer equipped with the Wiley 139 Mass Spectral data base with 139,859 spectra.

For chromatographic separation a 0.25 mm x 30m, 0.25 µm film thickness, RTX(R) -5 column (Restek Corporation) was used: injector temperature 220°C, oven temperature (programmed) 60-240 °C at 3°C/min, with a total run time 65 min, carrier gas He at a linear velocity of 32 cm/s. Injections of 1 µl were carried out in the split mode at a ratio of 1:20.

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