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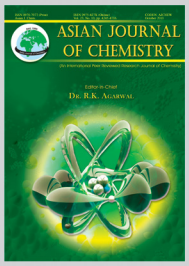

Composition of the Essential Oil, Neutral Volatile Oil and Petroleum Ether Extract from *Allium sativum* of Different Regions in Korea and Antioxidant Activity

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The composition of the essential oil, neutral volatile oil and petroleum ether extract from the bulbs of *Allium sativum* of two different regions in Korea were analyzed and identified by gas chromatographic-mass spectrometry (GC-MS). The essential oil yields (mL/100 g dry weight) were for the Yeongcheon (I) 0.40 % and Uiseong regions (II) 0.30 %, respectively. Twenty and twenty eight compounds comprising 97.91 and 84.95 % of the total peak area were identified in essential oil of Yeongcheon and Uiseong regions, respectively. Twenty seven and twenty nine compounds comprising 87.45 and 99.42 % of the total peak area were identified in neutral volatile oil of Yeongcheon and Uiseong regions, respectively. Thirty one compounds each comprising 93.61 and 95.68 % of the total peak area were identified in petroleum ether extract of Yeongcheon and Uiseong regions, respectively. The major components of essential and neutral volatile oil were dimethyl trisulfide (DMTS), diallyl disulfide (DDS), trisulfide di-2-propenyl (TSDP), methyl 2-propenyl disulfide, 2-vinyl-[4*H*]-1,3-dithiin (2-VDT), 3-vinyl-[4*H*]-1, 2-dithiin (3-VDT), 3-vinyl-1,2-dithiocyclohex-5-ene. The major components of petroleum ether extract were dimethyl trisulfide, phenylacetylene-2-d, DDS, 3-VDT, 2-VDT, TSDP, 1-propene 3,3'-thiobis. The identity of components of essential oil, neutral volatile oil and petroleum ether extract was confirmed on the basis of retention time, mass and supplemented library of NIST, USA. The neutral volatile oil and petroleum ether extract constituents are identified for the first time in garlic bulbs. The essential oil, neutral volatile oil and petroleum ether extract from the garlic bulbs were investigated for scavenging of the diphenylpicrylhydrazyl (DPPH) radical activity and the results demonstrate that the essential oil and the neutral volatile oil showed remarkable activity than the petroleum ether extract. Thus in general, garlic has potential as a natural antioxidant and thus inhibits undesired oxidation process.

Key Words: *Allium sativum* L. Liliaceae, Essential oil, Neutral volatile oil and petroleum ether extract constituents, Different regions, Antioxidant activity.

INTRODUCTION

Garlic (*Allium sativum* L. Liliaceae) is an important crop for culinary purposes, its pungent flavour adding a special taste to food. In some traditional Chinese and Egyptian dishes, garlic is fried in vegetable oil before adding food, which imparts a special taste and smell to the dish[1]. Garlic oil is also important in the food and pharmaceutical industries. Garlic oil is used in food preservation, especially cured meat and pickles. Today garlic is used to help prevent heart disease, including atherosclerosis (plaque build up in the arteries that can block the flow of blood and possibly lead to heart attack or stroke), high cholesterol, high blood pressure and to improve the immune system. In the pharmaceutical industry, garlic oil is much used due to its adaptogenic, anticarcinogenic[2] and antithrombotic, antiplatelet aggregating properties[3]. Many publications have discussed the chemical composition of garlic oil using different techniques of extraction and identification[4,5]. Various preparation techniques of garlic have been used to isolate and finally identify its components that mainly consist of organosulfur compounds. Medicinally used garlic oil is mostly prepared by hydro-distillation of raw garlic homogenate. The garlic essential oil components consists of the diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide, methyl allyl disulfide, methyl allyl trisulfide, 2-vinyl-1,3-dithiin, 3-vinyl-1,2-dithiin and several other sulfides[6-9]. Ajoene: a potent antithrombic agent from garlic has been reported[10]. Although substantial data of its chemical composition is available, there are no complete previous phytochemical reports that have been recorded for *Allium sativum* from Korea. In the present work, we investigated for the first time the chemical composition of the neutral volatile oil and petroleum ether extract isolated from Korean garlic bulbs of two different regions. The essential oil, neutral volatile oil and petroleum ether extract from the garlic bulbs were investigated for scavenging of the diphenylpicrylhydrazyl (DPPH) radical activity. The main aim of this

study was therefore to determine the main constituents of the essential oil, especially neutral volatile oil and petroleum ether extract growing in Korean regions and to evaluate the anti-oxidant activity of both oils and petroleum ether extract.

EXPERIMENTAL

Domestic garlic bulbs were purchased from the local market in Seoul, Korea in November 2010.

Essential oil isolation: The bulbs of *A. sativum* (500 g) after crushing were subjected to hydro-distillation in Clevenger-type apparatus for a minimum of 5 h. The resulting essential oil were obtained in a yield of 0.40 and 0.30 % w/w of (I) and (II), respectively, after drying over anhydrous sodium sulphate and stored at 4 ºC until use.

Isolation of neutral volatile oil: Commercially available garlic was re-dried at 45 ºC for 4 h. The bulb (1.5 kg) was crushed and extracted thrice with dichloromethane (1.5 l) at room temperature for 24 h to give 33.4 g of the extract. The extract (33.4 g) was hydro-distilled for 8 h with Clevenger's apparatus. The resulting volatile oil (1.8 g, of dried bulb) was re-dissolved in diethyl ether and successively washed with 5 % sodium bicarbonate, 1N NaOH, 1N HCl and brine. The remaining ethereal solution was dried with sodium sulphate and concentrated under reduced pressure to give 0.6 mL of a neutral volatile oil. The neutral oil was obtained as per the procedure[11].

GC-MS analysis of essential oil and neutral volatile oil: Samples of essential oil were diluted in hexane (spectroscopic grade) and analyzed in a Finnigan Focus GC/ Finnigan Focus DSQ MS system (Thermo Co., Germany) apparatus equipped with VB-WAX bonded PEG capillary column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness). Helium (1 mL/min) was used as a carrier gas. Sample volume was injected in the split mode 10 µL (split less). The injector was kept at 150 ºC. The column was maintained at 50 ºC for 10 min and then programmed to 200 ºC at 2 ºC and held for 0.5 h at 200 ºC. Detector temperature was held at 250 ºC. The MS was operated in EI mode at 70 eV in the m/z range 25-350. The identification of the compounds was performed by matching their recorded mass spectra of the GC-MS data system. Quantitative data were obtained from electronic integration peak areas and comparing their retention time and mass spectra library with those found in the literature and supplemented by the Wiley (Wiley 7th mass spectral library) & NIST MS Search 2.0 (National Institute of Standards and Technology) GC-MS libraries.

Preparation of petroleum ether extract: The bulbs of *A. sativum* (250 g) after drying in oven at 45 ºC for 4 h, after crushing immersed in petroleum ether (500 mL, 35-60 ºC) for overnight at room temperature and then the supernatant was concentrated under vacuum to yield (1.25 g) of the extract, which was small sample dissolved in hexane (spectroscopic grade) and prepare sample after filtration for GC-MS analysis.

GC-MS analysis of petroleum ether extract: Samples of petroleum ether extract were diluted in hexane (spectroscopic grade) and analyzed in a Finnigan Focus GC/ Finnigan Focus DSQ MS system (Thermo Co., Germany) apparatus equipped with Vesteck rtx-50 capillary column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness). The other conditions are same as in case of essential oil.

DPPH-radical-scavenging activity: The antioxidant activity of the two varieties of garlic essential oil, neutral volatile oil and petroleum ether extract based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{-}$) free radical, was determined by the method described[12]. The different concentrations (50 and 100 µg) of the tested samples (0.05 mL; extracts and tocopherol) were taken in different test tubes with 4 mL of a 0.006 % MeOH solution of $DPPH^{-}$. Water (0.05 mL) in place of the oil was used as control. Absorbance at 517 nm was determined after 0.5 h. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula, percentage radical scavenging activity = $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the extract /standard.

RESULTS AND DISCUSSION

Chemical constituents of the essential oil: The constituents identified by GC-MS analysis in order of elution of VB-WAX bonded capillary column are presented in Table-1. The oil was dominated by variety of sulfides. The major components of Yeongcheon and Uiseong regions were dimethyl trisulfide (7.70, 2.40 %), diallyl disulfide (45.56, 15.69 %), trisulfide di-2-propenyl (31.16, 58.97 %), respectively. However, the comparison of present results with literature[3,4,6] shows some qualitative and quantitative differences in the composition of garlic oil. The identification of the compounds was performed by matching their recorded mass spectra of the GC-MS data system. Quantitaive data were obtained from electronic integration peak areas and comparing their retention time and mass spectra library with those found in the literature and supplemented library. Other methods of identification and took help of by comparing mass data with data of library[13,14].

Chemical constituents of neutral volatile oil: The constituents identified by GC-MS analysis in order of elution of VB-WAX bonded capillary column are presented in Table-2. The oil was dominated by variety of sulfides. The major components of Yeongcheon and Uiseong regions were acetic acid butyl ester (2.46, 2.63 %), 1,3-dithiane (1.28, 2.92 %), methyl 2-propenyl disulfide (4.55, 8.55 %), methyl-*t*-propenyl disulfide (1.51, 2.26 %), dimethyl trisulfide (2.33, 7.85 %), diallyl disulfide (12.99, 17.34 %), 3-vinyl-[4*H*]-1,2-dithiin (14.36, 12.86 %), trisulfide di-2-propenyl (11.38, 5.47 %), 3-vinyl-1,2-dithiocyclohex-5-ene (32.34, 14.48 %), respectively. The identification of compounds is same as in essential oils. The chemical constituents of neutral volatile oil are reported for the first time in *A. sativum* bulbs.

Chemical constituents of petroleum ether extract: The constituents identified by GC-MS analysis in order of elution of Vesteck rtf-50 capillary column are presented in Table-3. The oil was dominated by variety of sulfides. The major components of Yeongcheon and Uiseong regions were disulfide methyl 2-propenyl (2.02, 2.61 %), phenylacetylene-2-d (3.29, 1.98 %), diallyl disulfide (14.28, 29.48 %), trisulfide methyl 2-propenyl (3.42, 1.72 %), 3-vinyl-[4*H*]-1,2-dithiin (18.95, 18.22%), 2-vinyl-[4*H*]-1,3-dithiin (21.00, 19.89 %), trisulfide di-2-propenyl (5.35, 4.60 %), respectively. The identification

TABLE-1
COMPOSITION OF KOREAN *Allium sativum* BULBS ESSENTIAL OIL FROM TWO DIFFERENT REGIONS

Retention time	Compounds	Yeongcheon regions	Uiseong regions
6.21	1-Propene 3,3'-thiobis	0.58	–
6.46	(2S, 2'S)- 2,2'-*bis*[1,4,7,10,13-pentaoxacyclopentadecane]	–	0.14
12.18	Methyl 1-propenyl disulfide	1.02	0.50
13.29	Methyl 2-propenyl disulfide	3.65	0.89
13.85	1,3-Dithiane	0.46	0.13
19.19	Dimethyl trisulfide (DMTS)	7.70	2.40
22.06	α-Thujone	–	1.88
23.21	Acetic acid (propylthio)-methyl ester	3.16	–
23.31	Cyclohexaneethanethiol acetate	–	0.62
25.06	Diallyl disulphide (DDS)	45.56	15.69
27.64	Camphor	–	0.21
32.08	Isobornyl acetate	–	0.29
32.25	Methyl 2-propenyl trisulfide (MPTS)	0.45	0.12
33.64	3-cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)	–	0.27
36.23	Butyl propenyl Sulfide	0.44	0.19
38.69	Dipropyl trisulfide (DPTS)	0.08	–
38.81	Diallyl tetrasulphide (DATS)	0.06	–
39.90	S-Oxodiallyl disulfide	0.44	0.23
40.44	2-Formyl-5-methylthiophene	–	0.21
44.98	Trisulfide di-2-propenyl	31.16	58.97
46.14	2-Vinyl-[4*H*]-1,3-dithiin (2-VDT)	1.42	0.99
48.38	Benzo thiophene octahydro-2-methyl (2α, 3αα, 7α α)-	0.35	0.28
48.59	u.i.	–	0.06
50.26	6-Nonenoic acid, methylester	–	0.10
51.24	7α-Hydroxyperhydroisobenzofuranone	0.25	0.21
52.27	Caryophyllene oxide	–	0.09
55.58	u.i.	–	0.06
61.28	17-Norkaur-15-ene, 13-methyl- (8α13α)-	–	0.08
62.26	Phenol, 2-methoxy-4-(2-propenyl)	0.08	–
65.15	1-Methoxy-3-trimethylsilyloxymethyloctane	0.12	0.10
65.37	u.i.	–	0.05
65.39	1-Methoxy-3-trimethylsilyloxymethylheptane	0.07	–
71.75	2(3*H*)-Naphthalenone 4,4α,5,6,7,8-hexahydro-4α-phenyl-(R)-	0.86	0.06
95.61	4α-Isopropyl-7α-methyltetracyclotetradeca-1,10-dien-12-one	–	0.13

–: Not detected.

of compounds is same as in essential oils. This is the first report of identified components in petroleum ether extract of garlic bulb.

DPPH-radical-scavenging activity: The free radical-scavenging activity of the essential oil, petroleum ether extract and the neutral volatile oil from the dichloromethane extract was tested through DPPH- method[12] and the results were compared with tocopherol. DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants. The method is based on the reduction of methanolic DPPH-solution in the presence of a hydrogen donating antioxidant, due to the formation of the non-radical form DPPH-H by the reaction. The oil was able to reduce the stable radical DPPH- to the yellow-coloured diphenylpicrylhydrazine. It has been found that cysteine, glutathione, ascorbic acid, tocopherol, polyhydroxy aromatic compounds (*e.g.*, hydroquinone, pyrogallol, gallic acid) and aromatic amines (*e.g.*, *p*-phenylene diamine, *p*-aminophenol), reduce and decolorize 1,1-diphenyl-2-picrylhydrazyl by their hydrogen donating ability[15]. In this present study essential oil and neutral volatile oil from dichloro methane extract from garlic bulbs showed a remarkable antioxidant activity than the petroleum ether extract, one of the possible mechanisms is polyphenolic-associated compounds (formation of non-extractable complex between high molecular weight phenolics and polysaccharides). Those kinds of phenolic compounds show antioxidant activity due to their redox properties, which play an important role in absorbing and neutralizing free radicals, quenching singlet and triple oxygen or decomposing peroxide. The essential oil, petroleum ether extract and neutral volatile oil from dichloro methane extract exhibited a concentration-dependent antiradical activity by inhibiting DPPH- radical (Fig. 1). The essential oil concentration of 100 µg exhibited highest free radical scavenging activities *i.e.*, above 90 %, followed by the neutral volatile oil from the dichloro methane extract which exhibited above 70 % at 100 µg concentrations, the activity was low in the petroleum ether extract, it exhibited only 55 % at 100 µg concentration. The DPPH activity of tocopherol showed higher degree of free radical-scavenging activity than that of both the oils and extract at each concentration points. Similar to our results Bozin *et al.*[16] reported that lower concentration of rosemary and sage essential oils exhibited highest antioxidant activity. Yin *et al.*[17] reported that organosulfur compounds derived from the garlic extract exhibited non-enzymatic antioxidant activity. Dandlen *et al.*[18] also reported that higher concentration of essential oil from thyme species exhibited highest antioxidant activity.

TABLE-2
COMPOSITION OF KOREAN *Allium sativum* BULBS NEUTRAL ESSENTIAL OIL FROM TWO DIFFERENT REGIONS

Retention time	Compound	Yeongcheon regions	Uiseong regions
4.43	Dimethyl disulfide (DMS)	0.54	–
4.58	Acetic acid butyl ester	2.46	2.63
5.24	6-Methyl-2-heptyne	0.32	0.92
5.39	4-Octyne-3,6-diol	–	2.03
6.41	1-Propene,3,3'-thiobis	0.79	3.75
10.34	Methyl propyl disulfide	–	1.28
11.56	3,4-Dimethylthiophene	0.66	0.45
12.21	1,3-Dithiane	1.28	2.92
13.64	Methyl-2-propenyl disulfide	4.55	8.55
13.96	Methyl-*t*-propenyl disulfide	1.51	2.26
19.32	Dimethyl trisulfide (DMTS)	2.33	7.85
25.31	Diallyl disulphide (DADS)	12.99	17.34
28.40	1-Hexanol 2-ethyl	–	1.15
28.71	2,2-Dimethylpropionic acid nonyl ester	–	0.29
29.06	1-Ethoxy-4,5-di-*t*-buytl-cyclohex-1-enedi-3,6-one	–	0.23
33.94	3-Methyl-1-phenylbutene	0.07	–
36.36	Disulfide di-2-propenyl	0.26	–
36.51	Sulfide butyl propenyl	–	0.97
37.16	Tetrahydro-thiopyran-3-carboxaldehyde	0.09	–
37.44	1-Methoxy-1-methyl-1-silacylcopentane	–	0.59
39.20	4*H*-Pyran-4-one, 3, 5-dihydroxy-2-methyl	0.22	–
40.48	3-vinyl-[4*H*]-1,2-dithiin	14.36	12.86
41.08	Trisulfide di-2-propenyl	11.38	5.47
46.73	3-Vinyl-1,2-dithiocyclohex-5-ene	32.34	14.48
48.00	Phenol, 2-Methoxy	–	1.78
48.59	1-Acetyl-3(α-bromobenzyl)-3-methoxypiperazine-2,5-dione	0.10	0.61
49.11	Cyclopropanecarboxylic acid, 2-pentyl methyl ester	0.07	–
49.39	2-Hexenoic acid 2-methyl	–	0.81
50.77	3-(2,2-Dimethylpropylidene) bicylco[3.3.1]nonane-2,4-dione	0.16	0.68
52.74	Dodecane	0.13	0.20
55.37	Ethyl 2-(3-thienyl) propanoate	0.12	0.59
56.19	Peracetyl ketone oxime derivative of 1,4,6-tri-o-methyl-2-hexulose	–	0.23
57.32	D-gala-1-ido octonic amide	0.12	–
62.24	Eugenol	0.14	1.95
65.23	5-(3-Phenyl-propenyl)-dihydrofuran-2-one	–	0.19
65.84	S-Oxodiallyl disulfide	0.18	–
71.71	2(3*H*)-Naphthalenone 4,4α,5,6,7,8-hexahydro-4α-phenyl-	0.16	–
72.03	Dimethyl 3,3,4-trimethyl-1,4-pentadiene-1,2-dicarboxylate	–	6.36
72.38	8-Methyl-2-methylthioindolizine	0.12	–

–: Not detected.

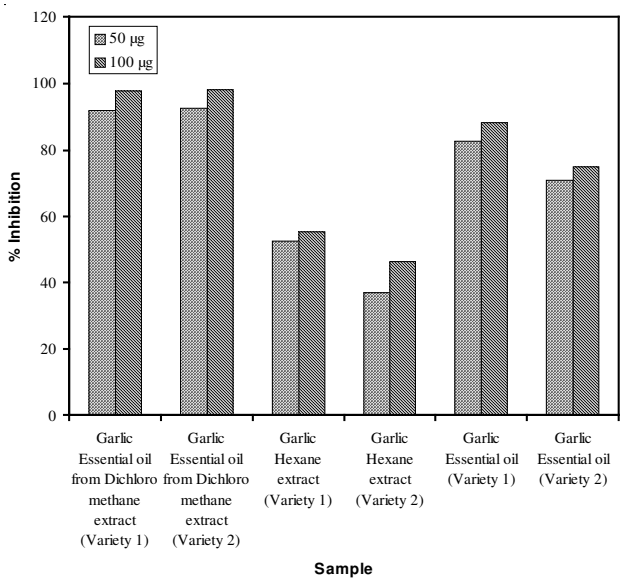

Fig. 1. Antioxidant activity of extracts from two varieties of garlic measured as percentage inhibition of DPPH radical

Conclusion

However, the comparison of present results showed some qualitative and quantitative differences in the composition of essential and neutral volatile oil. The petroleum ether extract also showed a variety of sulfides compounds similar to both the oils and differences in the chemical composition. The essential and neutral volatile oil showed a good antioxidant activity and therefore it can be used as an herbal medicine. The plant is not known to be toxic because it has been consumed by mankind for centuries without showing any sign of toxicity. Thus in general, garlic has potential as a natural antioxidant and thus inhibits unwanted oxidation process.

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TABLE-3
COMPOSITION OF KOREAN *Allium sativum* BULBS PETROLEUM ETHER EXTRACT EXTRACT FROM TWO DIFFERENT REGIONS

Retention time	Compound	Yeongcheon regions	Uiseong regions
3.46	Carbonic acid decyl ethyl ester	0.44	–
4.29	7-Methyl-tetracycloheptane	0.60	–
4.30	1-Propene 3,3'-thiobis	–	0.70
4.46	Decane	0.18	–
4.60	5,5-Dimethylpyrazolidine-3-one	–	0.14
4.90	Thiophene 2,4-dimethyl	0.39	–
5.07	Disulfide methyl 2-propenyl	2.02	2.61
5.27	1,3-Dithiane	0.59	0.37
5.46	u.i.	0.62	–
5.46	Dichloroacetic acid allyl ester	–	0.08
5.86	Trisulfide dimethyl	1.27	0.13
6.11	Phenylacetylene-2-d	3.29	1.98
6.44	Diallyl disulphide	14.28	29.48
6.87	2-Methyl-1-thiacyclohept-2-ene 1-oxide	0.28	–
6.88	Phenol 2-methoxy	–	0.94
7.01	2-Propeonic acid 2-ethylhexyl ester	0.82	–
7.22	Trisulfide methyl 2-propenyl	3.42	1.72
7.41	2-ethylidene [1,3]dithiane	–	0.80
7.42	Propanoic acid 3-mercapto-2-(mercaptomethyl)-	3.22	–
7.54	Methyl 2-diazo-4-(1-methyl-1-cyclohexyl)-3-oxobutanoate	0.27	–
7.86	3-Vinyl-[4*H*]-1,2-dithiin	18.95	18.22
8.27	2-Vinyl-[4*H*]-1,3-dithiin	21.00	19.89
8.54	Trisulfide di-2-propenyl	5.35	4.60
8.78	1-Propene 3,3'-thiobis	6.16	4.41
8.96	S-oxodiallyl disulfide	1.63	0.47
9.10	Eugenol	–	0.47
9.42	4-chloroorcinol	1.23	–
9.48	Thiophene 2-butyltetrahydo-	–	0.51
9.89	Phenol 2,6-*bis* (1,1-dimethylethyl)-4-methyl	0.23	–
10.45	Desulphosinigrin	–	0.07
11.02	Cyclopentadieno-9,10-phenanthrene	–	0.17
11.18	1,3-Dithiolane-2-propanol 2-methyl	0.62	–
11.41	6,10-Dithiaspiro[4.5]decan-1-ol	0.29	–
13.14	9(1*H*)-Phenanthrone 2,3,4,4α-tetrahydro-1α,4αα-dimethyl	–	3.55
13.53	u.i.	0.39	–
13.54	u.i.	–	0.34
13.69	N,N,-Dimethyl(4-nitro-1*H*-inden-1-ylidene)methanamine	–	0.42
13.70	3-Methoxymethoxy-2,3-dimethylundec-1-ene	0.78	–
14.11	3-Methoxymethoxy-2,3-dimethylundec-1-ene	0.29	–
14.40	1,3,5-Trimethyl-5-(pent-2-yl) barbituric acid	–	0.12
15.65	1,5-Dithiaspiro[5.6]dodecan-7-ol	o.32	–
15.93	8-Vinyltrithiatricyclodecane	–	0.20
16.10	Piperidine-3,4-didehydro-2,2,6,6-tetramethyl-4-(4-hydroxyphenyl)	–	0.08
16.30	Methyl 2, 6-anhydro-3, 4, 7-tridesoxy-1-erythro-hep-2-enulonate	2.43	–
16.32	1,2-Dimethyl-3-phenylcyclopropene(E)-1-	–	1.37
16.64	3-Isopropyl-4-methyl-dec-1-en-4-ol	1.81	–
16.65	8-Pentadecanol	–	0.92
18.65	1-Ethynyl-3 *trans* 91,1-dimethyl)-4, cismethoxycyclohexan-1-ol	–	0.55
19.27	1-Hexadecanol 2-methyl	–	0.11
23.27	Pentatriacontane	0.44	–
23.29	Dotriacontane	–	0.26

–: Not present.

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