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Original Article

Memory-Enhancing Properties of *Hypericum Scabrum* Essential Oil in a Rat Model of Dementia

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Abstract

Hypericum species are known to be used in traditional therapies. *H. scabrum* L. is one of the *Hypericum* species distributed in Turkey. In this study, we evaluated the effects of *Hypericum scabrum* essential oil inhalation on spatial memory in scopolamine-induced amnesic rats. The essential oil was characterized by GC-FID and GC-MS system. Male wistar rats were divided into 6 groups: control; scopolamine-alone treated; diazepam-treated (positive control); tramadol-treated (positive control); scopolamine with *Hypericum scabrum* essential oil 1%-treated; and scopolamine with *Hypericum scabrum* essential oil 3%-treated group. All rats were tested behaviorally to evaluate spatial memory peformances in Y-maze task and radial-arm maze task. The most abundant component of the essential oil was identified as α-pinene (51.3%). *Hypericum scabrum* essential oil application significantly improved spatial memory as compared to scopolamine-alone treated rats as evidenced by increases in spontaneous alternation behavior in Y-maze task and decreases in the number of working memory errors and reference memory errors in the radial-arm maze task in this study. *Hypericum scabrum* essential oil inhalation could be used as a complementary therapy to reduce memory impairments in the patients with dementia and related diseases.

Keywords: Alpha-pinene, *Hypericum scabrum* essential oil, memory, radial-arm maze, scopolamine, Y-maze.

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1. Introduction

Dementia is a complex and multifactorial syndrome, causing an increasing global concern. About 46 million people has been diagnosed with dementia worldwide; unfortunately, this number is anticipated to rise to 131.5 million by 2050 [1]. Dementia is defined as cognitive impairment in more than one cognitive area including learning and memory [2]. The patients with Alzheimer's disease (AD) which is the most common type of dementia accounting for 50–60% of the total cases of dementia, show degeneration in hippocampus and amygdala regions of the

brain [3]. Therefore, memory impairment in these patients is almost inevitable.

This age-related progressive syndrome is also responsible from the caregiver burden and high health care costs. Unfortunately, no effective treatment has been developed against dementia so far. Being the only top 10 cause of disability, AD does not even have a therapy to slow its progression [4]. However, there is evidence that preventive therapies may help to reduce the risk of AD development. Epidemiological studies suggest that diet and lifestyle factors are strongly related to onset and progress of AD [5].

The genus *Hypericum* L. is the largest member of the Clusiaceae Lindl. (syn. Hypericaceae Juss.) which includes 484 species [6]. *Hypericum* species are known to be used in traditional therapies, and many of them have been utilized for their economic significance as natural sources. *H. perforatum* L., for instance, commonly known as St. John's Wort, stands as a natural medicine in the treatment of depression. *H. perforatum* is one of the important dietary supplements sold in European market [7].

In the Flora of Turkey, a total of 96 *Hypericum* species are present, 46 of which are endemic. In the traditional Turkish medicine, *Hypericum* species are used for the treatments of skin injuries, diarrhea and ulcers [8].

H. scabrum L. is one of the *Hypericum* species distributed in Turkey. It is also an important species of Traditional Chinese Medicine. It is reported to be used against numerous disorders such as ailments of the liver, gall bladder, intestines, heart, and systitis. Recently, a few compounds of this species have been shown to exhibit moderate hepatoprotectant and antidepressant activities [9].

Essential oils (EO) obtained from plants consisting of mainly bioactive monoterpenes may have attractive or repellent effects. *H. scabrum* was among these plants of which EO was reported to possess insecticidal activity [10]. Insecticidal effect results from the inhibition of acetylcholinesterase (AChE) which hydrolyzes the neurotransmitter acetylcholine (ACh) [11]. The inhibition of AChE also takes part in symptomatic treatment of AD [12]. In fact, AChE inhibitors are the main medications in the current strategy of AD treatment. Therefore, we assumed that EO of *H. scabrum* could improve memory in laboratory animals. Scopolamine-induced dementia was used as an animal model in this study. Scopolamine, an antagonist of muscarinic ACh receptors, has been used to induce learning and memory problems resembling the symptoms seen in AD [13]. In this study, we evaluated memory performances of rats exposed to *H. scabrum* EO by inhalation in scopolamine model of AD type dementia.

2. Materials and Methods

2.1. Plant Materials and Essential Oil Preparation

H. scabrum plant samples were harvested in Elazig, Eastern Anatolia, Turkey. Aerial parts (stem, leaves and flowers) of the samples

were used in this research to obtain the essential oil. The authors identified the samples of the plants and a voucher specimen was registered (FUH.8386) and saved in the Firat University Herbarium (FUH) as a reference. The oil was obtained by hydro-distillation for 3 h using a Clevenger-type apparatus. The total essential oil yield was 0.6 % (v/w).

2.2. Gas Chromatography (GC-FID) Analysis

GC- FID analysis of the *H. scabrum* essential oil was performed in Plant Products and Biotechnology Research Laboratory (BUBAL), Firat University, using Hewlett Packard-Agilent 5973N GC-MS system with 6890 GC equipped with a flame ionization detector (FID). HP-5 MS column (30 m×0.25 mm i.d., film thickness (0.25 μm)) was used with helium as the carrier gas. The injection volume was 1.0 μL of diluted solution (1/100) of oil in *n*-hexane. Injector temperature was 250°C, and the flow rate was 1.3 mL/min. (splitless mode). The GC oven temperature was kept at 70°C for 2 min and programmed to 150°C at a rate of 10 °C/min and then kept constant at 150°C for 15 min to 240°C at a rate of 5°C/min. The percentage composition of the essential oils was computed from GC–FID peak areas without correction factors.

2.3. Gas Chromatography (GC-MS) Analysis

The column and analysis conditions of GC-FID and GC-MS were the same. Calculation of retention indices (RI) was done by using a series of *n*-alkanes as reference points. MS were taken at 70 eV and a mass range of 35–425. The identification of the compounds was based on comparison of their retention indices (RI), and mass spectra with those obtained from authentic Wiley (7th version) and Nist 98 libraries.

2.4. Animals

42 male Wistar rats weighing 250 ± 50 g were used in this study. The animals were kept in a temperature and light-controlled room (22°C, a 12-h cycle). Regular laboratory food pellets for rodents were used to feed the animals. Food and water were given *ad libitum*. 6 group of animals, 7 rats in each group, were used: (1) 0.9% saline with 1% Tween 20 treatment was applied to the control group; (2) Scopolamine (Sco) with 0.9% saline and 1% Tween 20 treatment was applied to scopolamine-alone-treated group, as negative control; (3) Diazepam (DZP, 1.5 mg/kg) with 0.9% saline and 1% Tween 20 treatment was applied to diazepam alone-treated group only in elevated plus maze task, as positive control; (4) Tramadol (TRM, 10 mg/kg) with 0.9% saline and 1% Tween 20 treatment was applied to tramadol-alone treated group only in forced swimming test, as positive control; (5) *H. scabrum* essential oil 1% (Sco+HEO1%) was subjected to scopolamine-induced rats and (6) *H. scabrum* essential oil 3% (Sco+HEO3%) was subjected to scopolamine-induced rats. Only 5th and 6th groups received essential oil. Rats were treated in accordance with the guidelines of the animal bioethics of the Act on Animal Experimentation and Animal Health and Welfare from Turkey and all procedures were in compliance with Directive

2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. This study was approved by the Committee on the Ethics of Animal Experiments of the Firat University (Permit Number: 213, 30.12.2015) and also, efforts were made to minimize animal suffering and to reduce the number of animals used.

2.5. Inhalation Apparatus and Drug Administration

Inhalation of essential oil by rats was carried out within a Plexiglas chamber (50 x 40 x 28 cm). Experimental rats were exposed to *H. scabrum* essential oil diluted with 1% Tween 20 (v/v) and exposed (200 µL, either 1% or 3%) via an electronic vaporizer (Oregon Scientific WS113) placed at the bottom of chamber, but out of reach of the animals [14]. In another chamber, control and scopolamine alone-treated animals, were exposed to 0.9% saline with 1% Tween 20 solution. 1% of essential oil was selected as a normal dose used in aromatherapy and a higher concentration (3%) in order to emphasize the effects [15]. Rats in the *H. scabrum* essential oil groups were exposed to oil vapors for controlled 15 min period, daily, for 21 continuous days. Simply, the rats inhaled the essential oil vapor within the chamber [16] [17]. This method allows shorter therapeutic response onset time. An inhalation chamber enables in vivo studies by mimicking clinical use of the drug [18]. Chambers were always cleaned up (10% ethanol solution). Scopolamine hydrobromide (Sigma-Aldrich, Germany) was used as negative control and was dissolved in an isotonic solution (0.9% NaCl) and 1.5 mg/kg scopolamine was injected intraperitoneally (*i.p.*), 30 min before the behavioral testing. Diazepam (Sigma-Aldrich, Germany) and tramadol hydrochloride (Sigma-Aldrich, Germany) were used as positive controls and were injected *i.p.* in a volume of 1 ml/kg in laboratory rats, 1 h before behaviorally tested.

2.6. Y-Maze Test

In the Y-maze task short-term memory was evaluated by spontaneous alternation behavior. The Y-maze test selected in the current study comprised of three arms (35 cm long, 25 cm high and 10 cm wide) and an equilateral triangular central space. After 15 min of *H. scabrum* essential oil (HEO1% and HEO3%) inhalation, rats were positioned at the end of one arm and permitted to move without obstruction through the maze for 8 min. The hind paws of the rat were had to be completely within the arm to be counted as an arm entry. Entries into all three arms such as ABC, CAB or BCA were defined as spontaneous alternation behavior. In this regard, the number of maximum spontaneous alternation behaviors was equal to the entered total number of arms minus 2 and spontaneous alternation percentage was obtained as (actual alternations/maximum alternations) X 100. Spontaneous alternation behavior is considered to reflect short-term memory, namely spatial working memory. The Y-maze was cleaned with a solution of 10% ethanol and dried with a paper towel after each trial.

2.7. Radial Arm-Maze Test

The radial arm-maze used in the current study contained eight arms, numbered from 1 to 8 (48 cm x 12 cm), extending radially from a main area (32 cm in diameter). The device was positioned 50 cm above the floor. Various visual cues were positioned around the maze in the laboratory at the same position during the study. There was a food cup consisting of a single 50 mg food pellet at the terminal of each arm. One week prior to the performance of the maze task, a restricted diet was applied to the rats in order to reduce their body weight to 85% of their original weight, with water always being available *ad libitum*. Before the actual training started, three or four rats were put together in the radial arm-maze and permitted to explore for 5 min and take the food without restrictions. The food was available throughout the maze at the beginning, but the number of food pellets decreased and finally only one food pellet was put in the food cup. The rats were trained for 4 days to explore the maze and eat the food. To evaluate the basal activity of rats in radial arm-maze, the rats were trained 5 times per day to explore the maze and eat the bait. The test ended for each rat when all 5 food pellets have been consumed. If they could not consume all of the pellets for 5 min, the test ended. After adaptation, all rats were trained with 1 trial per day. Briefly, 15 min after *H. scabrum* essential oil (HEO1% and HEO3%) inhalation, each rat was put independently in the center of the maze and subjected to working and reference memory tasks, in which same 5 arms (number 1, 2, 4, 5, and 7), were baited for each daily training trial. The other 3 arms (number 3, 6, and 8) were never baited. The selection of the baited arms is based on the fact that animals tend to solve the maze using a neighboring arm selection strategy. In this case, we altered neighboring arm patterning behavior by only baiting 5 arms (number 1, 2, 4, 5, and 7) exposing animals to alter their strategy and avoid the unbaited arms. When all limbs of the rat were within an arm, then this was accounted as an arm entry. A working memory error was considered as entering an arm containing food, but entered previously, while a reference memory error was considered as entering an arm that was never baited. Reference memory is regarded as a long-term memory for information that remains continuous over repetitive trials (memory for the locations of baited arms), whereas working memory is considered a short-term memory in which the information that the rats had to remember changes in each trial. The maze was cleaned with a 10% ethanol solution and dried with a paper towel after each trial.

2.8. Statistical Analysis

One-way analysis of variance (ANOVA) was used to analyze behavioral scores within Y-maze. Additionally, Tukey's post hoc test using Graph Pad Prism 6 software was used to evaluate differences between groups. On the other hand, for radial arm-maze test, two-way ANOVA was used. All results are expressed as mean ± standard error of mean (S.E.M). F values for which $p<0.05$ were considered as statistically significant.

3. Results and Discussion

3.1. Chemical Composition of the Hypericum scabrum Essential Oil

Chemical composition of *Hypericum scabrum* EO is presented in Table 1. A total of 68 different compounds were identified in *H. scabrum* essential oil, constituting 95.2% of the total oil. Monoterpene hydrocarbons were found as the most abundant components (72.4%), followed by sesquiterpene hydrocarbons (16.4%). Other hydrocarbons were identified to be present in the oil as 4.5%, while fatty acids composed 1.5% of the oil. Furthermore, phytol, a diterpene alcohol, was identified in *H. scabrum* essential oil sample in this study.

The most abundant component of *H. scabrum* EO was identified as α-pinene (51.3%) in this study. The other major components were found as β-pinene (7.7%), spathulenol (3.4%), and *o*-cymene (3.0%). α-Pinene and β-pinene were among monoterpene hydrocarbons, while *o*-cymene is monoterpene hydrocarbon derivative. Spathulenol, on the other hand, is a tricyclic sesquiterpene alcohol.

In another study, the major compounds of *H. scabrum* EO from different location of Turkey were reported as α-pinene (9.3%), camphor (5.9%), terpinen-4-ol (5.1%), δ-cadinene (4.5%), pulegone (4.5%), and γ-muurolene (4.1%) [19]. Our high concentration of α-pinene was also determined as the major component in this study, however, its amount was much lower than it was found in our oil. On the other hand, EO composition from Iranian samples of *H. scabrum* indicated α-pinene (50.0%) as the most abundant component followed by β-pinene (9.7%), limonene (6.7%), carvacrol (5.8%) and (E)-β-ocimene (5.6%) [20]. In this sample, α-pinene and β-pinene concentrations were close to our results in our study. In another study, oil composition of *H. scabrum* samples from Iran revealed the major components as α-pinene (40.9%), spathulenol (7.9%), and β-pinene (5.2%) [21]. Another study on Iranian samples of *H. scabrum* EO indicated the major components as α-pinene (45.3%), *n*-nonane (5.6%), and thymol (5.3%) [22]. These data show that chemical composition of *H. scabrum* EO samples from Iran were quite similar to our results in this study. Especially, concentrations of the major component, α-pinene, in these studies were reported to be close to our results.

3.2. Effect of Hypericum Scabrum Essential Oil in Y-Maze Task

The first behavioral test used in this study was Y-maze task. In this task, spontaneous alternation percentage was evaluated in order to assess spatial memory performances of the tested animals. In this test, one-way ANOVA revealed significant overall differences between all groups [$F(3,12)=8.93$, $p<0.01$]. Additionally, Tukey's *post hoc* analysis was performed to compare differences between each group. This test revealed significant differences between control vs. Sco ($p<0.001$), Sco vs. Sco+HEO1% ($p<0.01$) and Sco vs. Sco+HEO3% ($p<0.01$). Non-significant differences were observed between Sco+HEO1% and Sco+HEO3% groups.

3.3. Effect of Hypericum Scabrum Essential Oil in Radial Arm-Maze Task

Radial arm maze task is another test to evaluate spatial memory in laboratory animals. In our study, we assessed working memory and reference memory performances of the tested rats. For working memory errors, two-way ANOVA revealed significant differences in time [F (6,112) =2.51, $p<0.05$] and significant differences in groups [F (3,112) =5.09, $p<0.005$]. For reference memory errors, two-way ANOVA revealed significant differences in time [F (6,112) =3.15, $p<0.01$] and significant differences in groups [F (3,112) =9.11, $p<0.0001$] (Figure 1 and 2).

Previously, many studies showed neuroprotective effects of *H. perforatum* extract [23] [24] [25] [26]. In the aforementioned studies, memory-enhancing activities of *H. perforatum* extracts were reported using different behavioral tasks. Additionally, *H. scabrum* extract was recently showed to possess high antioxidant activity and reverse memory impairment induced by high fat diet [27].

Monoterpenes and especially α-pinene was reported previously to improve cognitive functions [28]. α-Pinene in our EO, therefore, could be the main component to improve cognition in laboratory rats. Many biological activities of α-pinene has been speculated previously, including gastroprotective [29], anti-cancer [30], antioxidant, anti-inflamatory, anti-nociceptive, antibacterial, antifungal [31], and anti-ulcerogenic activities [32]. α-pinene is also used in numerous industrial procedures including fragrance and flavor industry, color printing, and paint process [33]. Additionally, insect-repellant functions of α-pinene has been reported which could be related to acetylcholinesterase inhibitory activity [34].

Biological activities of the other major components found in *H. scabrum* essential oil, β-pinene, and spathulenol have also been reported previously. β-Pinene was shown to induce antidepressant effects in mice [35]. In addition, *Litsea glaucescens* essential oil was reported to possess antidepressant activities, in which β-pinene and linalool were the major components [36]. Spathulenol, on the other hand, was reported to have antioxidant, anti-inflamatory, and antiproliferative activities [37]. Therefore, β-pinene and spathulenol could possess neuroprotective activities.

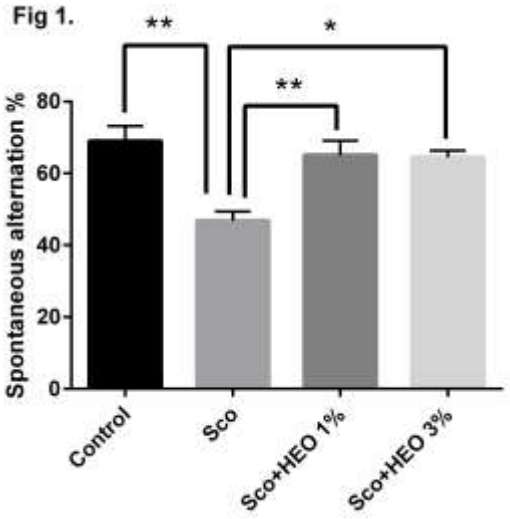

Figure 1. Effects of the inhaled Hypericum scabrum essential oil (HEO1% and HEO3%) in the Y-maze on the spontaneous alternation % in the scopolamine (Sco)-treated rats. Values are means ± SEM (n = 7 animals per group).

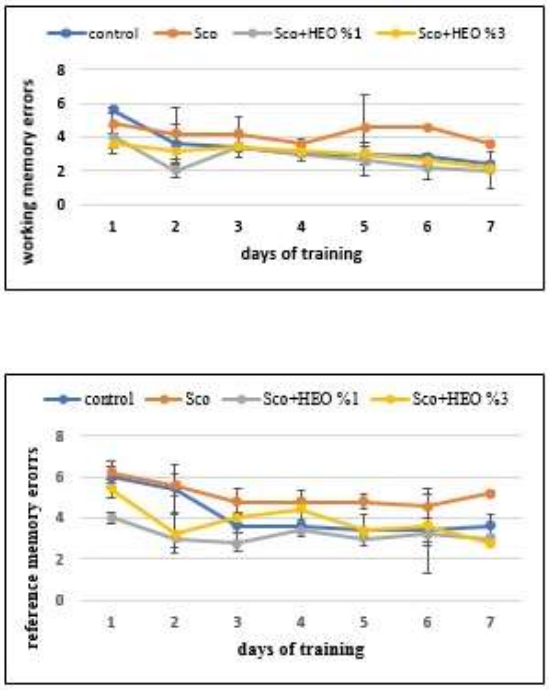

Figure 2. Effects of the inhaled *Hypericum scabrum* essential oil (HEO1% and HEO3%) on the working memory errors (a), and the reference memory errors (b) during 7 days training in the radial arm-maze in the scopolamine (Sco)-treated rats. Values are means ± SEM (n = 7 animals per group).

4. Conclusion

At present, there is no cure for AD. Although a number of medications are prescribed for the disease, those can only lower the symptoms without affecting the progress of AD. The most validated ones are cholinesterase inhibitors. Current approved cholinesterase inhibitors against AD are rivastigmine, tacrine, donepezil, and galantamine. Synthetic medications could be able to improve cognitive functions; however, most of them cause side effects which significantly reduce life quality of the patients. Therefore, more effective drugs with lower side effects are investigated throughout the world. The trend for traditional medicine in this decade can be understood for this reason [38].

Hypericum scabrum essential oil in this work exhibited memory enhancing activities in scopolamine-treated amnesic rats. High α-pinene concentration of the essential oil suggests neuroprotective effects of the compound. We think that more research on biological effects and possible mechanism of α-pinene is highly necessary for the further studies. The other major components, β-pinene and spathulenol, could be possible to contribute to the neuroprotective effects of the oil. Consequently, *Hypericum scabrum* essential oil could be used as a complementary therapy in order to reduce memory impairments in dementia.

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Table 1. Constituents of *Hypericum scabrum* essential oil.

NO	Components	RI	%	Method of identification
1	Nonane	995	1.6	RI, MS
2	α-Thujene	1015	0.9	RI, MS
3	**α-Pinene**	**1024**	**51.3**	RI, MS
4	α-Fenchene	1033	0.1	RI, MS
5	Camphene	1034	0.4	RI, MS
6	**β-Pinene**	**1055**	**7.7**	RI, MS
7	Sulcatone	1060	0.1	RI, MS
8	β-Myrcene	1064	2.4	RI, MS
9	α-Phellandrene	1076	0.1	RI, MS
10	Δ-3-Carene	1078	0.1	RI, MS
11	α-Terpinene	1085	0.1	RI, MS
12	***o*-Cymene**	**1091**	**3.0**	RI, MS
13	Limonene	1094	2.0	RI, MS
14	β-Phellandrene	1096	0.3	RI, MS
15	β–*Trans* ocimene	1099	0.1	RI, MS
16	*Cis*-ocimene	1107	1.5	RI, MS
17	γ-Terpinene	1116	0.5	RI, MS
18	α-Terpinolene	1136	0.2	RI, MS
19	Isopropenyltoluene	1140	0.1	RI, MS
20	Undecane	1147	1.4	RI, MS
21	Fenchol	1162	0.1	RI, MS
22	α-Campholenal	1167	0.2	RI, MS
23	*Trans*-pinocarveol	1177	0.1	RI, MS
24	*Trans*-Verbenol	1180	0.2	RI, MS
25	Borneol	1199	0.1	RI, MS
26	4-Terpineol	1204	0.2	RI, MS
27	*m*-mentha-2,8-diene	1215	0.7	RI, MS
28	D-Verbenone	1223	0.1	RI, MS
29	Carvacrol	1295	0.1	RI, MS
30	α-Cubebene	1335	0.1	RI, MS
31	α-Longipinene	1338	0.1	RI, MS
32	Ylangene	1353	0.1	RI, MS
33	α-Copaene	1358	0.4	RI, MS
34	β-Bourbenene	1365	0.2	RI, MS
35	β-Elemene	1369	0.1	RI, MS
36	α-Gurjunene	1382	0.1	RI, MS
37	β-Caryophyllene	1392	1.5	RI, MS

NO	Components	RI	%	Method of identification
38	β-Cubebene	1399	0.2	RI, MS
39	Aromadendrene	1405	0.3	RI, MS
40	β-Farnesene	1414	0.1	RI, MS
41	α-Humulene	1416	0.1	RI, MS
42	Neoalloocimene	1420	0.1	RI, MS
43	γ-Cadinene	1429	1.7	RI, MS
44	Germacrene-D	1434	1.2	RI, MS
45	4,11-selinadiene	1436	0.1	RI, MS
46	β-Selinene	1439	0.3	RI, MS
47	γ-Muurolene	1441	0.5	RI, MS
48	α-Selinene	1444	0.3	RI, MS
49	α-Muurolene	1445	0.2	RI, MS
50	α-Farnesene	1448	0.1	RI, MS
51	α-Amorphene	1454	0.7	RI, MS
52	Δ-Cadinene	1457	1.1	RI, MS
53	L-Calamenene	1459	0.4	RI, MS
54	α-Cadinene	1469	0.2	RI, MS
55	α-Calacorene	1472	0.2	RI, MS
56	Dodecanoic acid	1485	0.5	RI, MS
57	**Spathulenol**	**1494**	**3.4**	RI, MS
58	Caryophyllene oxide	1497	1.1	RI, MS
59	γ-Gurjunene	1499	0.2	RI, MS
60	Isospathulenol	1525	0.3	RI, MS
61	γ-Selinene	1533	0.3	RI, MS
62	α-Cadinol	1538	0.7	RI, MS
63	12-Norcyercene-B	1557	0.5	RI, MS
64	Hexahydro formyl acetone	1630	0.2	RI, MS
65	*n*-Hexadecanoic acid	1692	1.0	RI, MS
66	Phytol	1791	0.4	RI, MS
67	9,12,15-octadecatriene-1-ol [ZZZ]	1809	0.4	RI, MS
68	Tricosane	1900	0.1	RI, MS
	TOTAL		**95,2**	