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Phytochemical Constituents and GC-MS Profiling of the Whole Plant Ethanol Extract of *Thesium viride* Hill and its Oral Toxicity in Balb/C Mouse Model

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Abstract

Thesium viride Hill, a member of the Santalaceae family, treats several ailments. However, few pharmacological investigations have been done to ascertain its folklore usage. The present study evaluated the presence of phytochemical constituents and Gas Chromatography-Mass Spectroscopy profiling of the whole plant extract of Thesium viride and its oral toxicity in the Balb/c mouse model. The whole plant-dried sample was collected from Zaria local government, Kaduna State, Nigeria, and 500 grams of the pulverised plant was extracted by the Soxhlet method using different solvents. Phytochemical screening and GC-MS analyses of the whole plant ethanol extract were done according to standard procedures. Acute oral toxicity studies of the extracts were carried out on BALB/c mice weighing 17-23g following recommendations from the OECD. The phytochemical analysis of Thesium viride whole plant extracts showed the presence of cardiac glycosides, carbohydrates, flavonoids, tannins, saponins, steroids, triterpenes, and alkaloids. In addition, the GC-MS analysis of the ethanol extract of Thesium viride revealed the presence of thirty-four bioactive compounds, the major ones being Bis (2-ethylhexyl) phthalate, cis-13-Octadecenoic acid, trans-13-Octadecenoic acid, n-Hexadecanoic acid, 9-Octadecenoic acid (Z), and Diethyl Phthalate with peak areas (concentrations) of 34.44%, 29.75%, 4.18%, 3.60%, 2.62%, and 2.01% respectively. The median lethal dose (LD50) for acute toxicity studies of different fractions of Thesium viride on Balb/c mice was greater than 5000 mg/kg. The study concludes that the whole plant extract of Thesium viride is rich in phytoconstituents with pharmacological prospects. The extract is practically non-toxic and safe when administered orally in mice.

Keywords: Acute toxicity; Balb/c mice; GC-MS profiling; Phytochemical screening; Thesium viride

INTRODUCTION

Thesium viride Hill, a member of the Santalaceae family, is primarily found in Africa, Asia, and Europe (Moore *et al.*, 2010). *Thesium viride* is a hemiparasitic subshrub up to 45 cm tall, with branching and two mm-thick greyish-green stems emerging from a woody base (Bosch, 2008). The leaves are simple and whole, sessile, narrowly oval to linear, 4 mm x 0.5 mm, acuminate, and alternate. Flowers are terminal and axillary, solitary, bisexual, regular, (4—) 5-merous, with oblong to narrowly ovate brackets, 2 mm long, with a sharp apex; the perianth tube is 1 mm in diameter. The ovary is superior, styles up to 3 mm long, and stamens are inserted on the perianth tube. The fruit has a persistent perianth 2 mm long and an ellipsoid, ridged achene about 3 mm x 2 mm and reticulate veins (Bosch, 2008). About 300 species of *Thesium* can be found in Africa and Eurasia. There are approximately 17 species in East Africa and 175 in Southern Africa. *Thesium* parasitises a variety of plants without clearly displaying host specificity. For the majority of the African species, there are not many specifics. The tiny flowers identifying each species make diagnosing challenging (Bosch, 2008)A variety of *Thesium* species are used in traditional African medicine. For instance, uterine infections are treated with *T. lacinulatum* roots. While the roots of *T. hystix* are used to treat kidney, bladder, and lung infections, those of *T. utile* are used to treat gastrointestinal diseases (Belakhdar*et al.*, 2014). To treat jaundice, Beninese people consume powdered aerial portions either alone or combined with a sauce, and they also drink a decoction made from the branches. It is recommended for the treatment of ulcers (Polhill, 2005). The plant's aerial part treats

jaundice, an enlarged liver, and splenomegaly (Iwu, 2014).

In Nigeria, *Thesium viride* is called "Huntu" by the Hausa ethnic group (Moore *et al.*, 2010). The plants have been proven traditionally effective against several ailments. The leaves are boiled in water as a decoction to treat jaundice and fever and as a laxative for intestinal worms. The extract and fractions of *T. viride* have been reported to demonstrate antimicrobial, antibacterial, and antiulcer and protect and improve the liver's antioxidant enzymes against CCl4-induced liver damage (Kamaruding *et al.*, 2020; Shehu *et al.*, 2016, 2022). A few pharmacological investigations have been conducted to ascertain its phytochemical profile and folklore usage (Shehu *et al.*, 2016, 2022)). However, there is a need to carry out a detailed phytochemical analysis and toxicity study to establish the important characteristics of the plant yet to be exploited by research. Hence the present study was conducted to evaluate the presence of phytochemical constituents and GC-MS profiling of the whole plant extract of *Thesium viride* and its oral toxicity in the Balb/c mouse model.

MATERIALS AND METHODS

Ethical Considerations

The study protocol and approval to do the study were obtained from the Ethical Committee on Animal Use and Care in Ahmadu Bello University (ABU) in Zaria, Nigeria, with approval number ABUCAUC/2021/078.

Collection and Identification of the Plant Material

The whole plant-dried T. viride (Plate I) sample was collected from Zaria local government, Kaduna State, Nigeria, in February 2020. Plant identification was made at the herbarium unit, Department of Botany, ABU, Zaria, with accession number ABU06986.

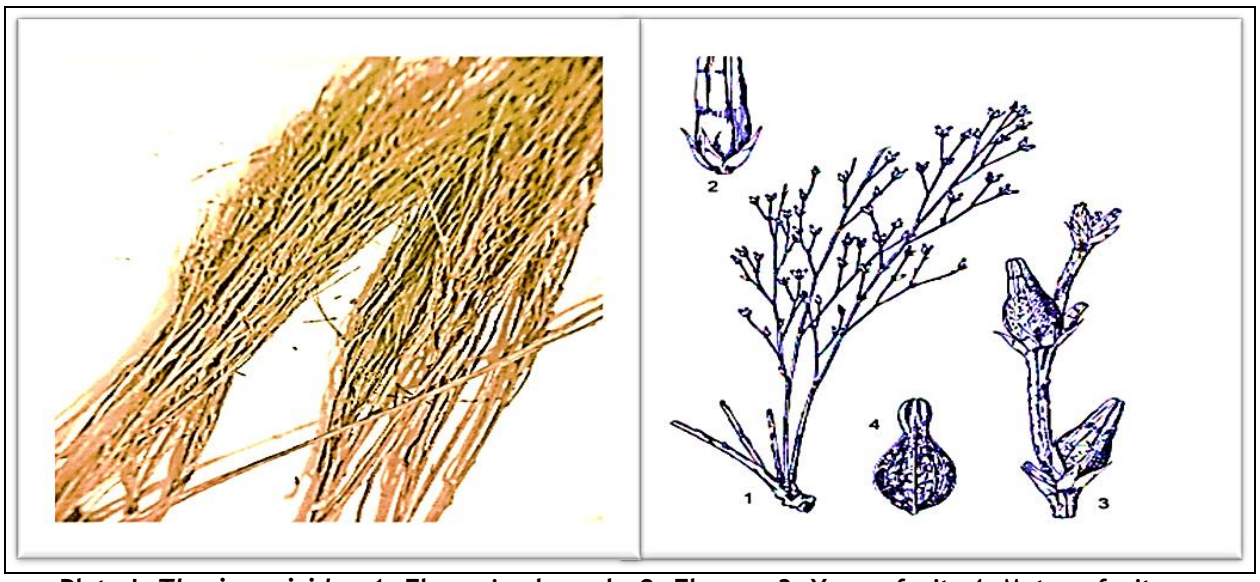

Plate I: *Thesium viride* – 1. Flowering branch; 2. Flower; 3. Young fruit; 4. Mature fruit

Preparation of Plant Extracts

Five hundred grams (500 g) of the powdered *Thesium viride* was soaked in 2 litres of 70% methanol for about 72 hours using cold maceration and stirring intermittently to produce the extract. The extracts were filtered, and the filtrate was concentrated at 40^0C and kept in a desiccator for further studies (Ayawa *et al.*, 2021). Phytochemical Evaluation

The qualitative phytochemical screening of the plant was carried out in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, ABU, Zaria. The presence of phytochemical constituents, including alkaloids, flavonoids, anthraquinone, carbohydrates, tannins, unsaturated steroids, triterpenes, cardiac glycosides, and saponin, were analysed according to standard methods (Harborne, 1973; Trease and Evans, 1996).

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC-MS analysis was done at the Multipurpose Laboratory, Department of Chemistry, ABU, Zaria, Nigeria, to determine the bioactive compounds of the whole plant of *Thesium viride*. The analysis was conducted on a GC-MS machine under standard operational conditions. The bioactive compounds with their retention times (RT), peak areas (%), and abundance were identified by comparison of the acquired spectra with those in the existing

National Institute of Standards and Technology (NIST) library.

Acute Oral Toxicity Study

Acute toxicity studies were carried out on twenty (20) BALB/c mice weighing 17–23 g and maintained in standard laboratory conditions following recommendations from the Organization for Economic Cooperation and Development (OECD) (423; OECD guideline, 2002). In the first phase of the oral toxicity test, the mice were distributed into five groups based on the fraction of two mice each and were given a single dose of 2000mg/kg of the various extract fractions after six hours of fasting. The animals were observed for 24 hours to monitor their behaviour, such as changes in agitation, convulsion, piloerection, stretch, tachycardia, defaecation, diarrhoea, abdominal writhing, tearing, cyanosis, sleepiness, tremor, ptosis, as well as mortality. Phase 2 involved using ten mice, which were distributed into five groups based on the fractions of two mice each. Each mouse was administered a higher dose of 5000 mg/kg of the various fractions and then observed for 24 hours for their behaviour and mortality. The LD_{50} value was calculated using the equation:

$LD_{50} = \sqrt{D0 \text{ X } D100}$

Where D0 = the highest dose that gave no mortality

D100 = lowest dose that produced no mortality

RESULTS

Phytochemical constituents and GC/MS profile of *Thesium viride*

Table 1 shows the qualitative phytochemical constituents of *Thesium viride* using different solvent extracts. The results show that all the extracts contain cardiac glycosides, carbohydrates, flavonoids, tannins, and saponins. Additionally, steroids, triterpenes, and alkaloids are present in all the extracts except in the ethyl acetate and aqueous fractions. Anthraquinones were absent in all the fractions (Table 1).

Table 1: Qualitative phytochemical constituents of *Thesium viride* using different solvent extracts

Compounds	Hexane Fraction	Ethanol Fraction	Ethyl acetate Fraction	Butanol Fraction	Aqueous fraction
Steroids & Triterpenes	+	+	-	+	+
Cardiac Glycosides	+	+	+	+	+
Anthraquinones	-	-	-	-	-
Alkaloids	+	+	+	+	-
Carbohydrates	+	+	+	+	+
Flavonoids	+	+	+	+	+
Tannins	+	+	+	+	+
Saponins	+	+	+	+	+

+ = Present, - = Absent

Furthermore, based on the results of the phytochemical screening, the ethanol fraction was further subjected to gas chromatography-mass spectrometry (GC/MS) profiling to determine the bioactive compounds. As indicated by the chromatogram of the GC/MS analysis of the ethanol extract of *Thesium viride*, the results show retention time, peak area (Figure 1A), and abundance of bioactive compounds (Figure 1B). Peak size is another parameter used to present the gas chromatography results. The peak measurement is carried out from the base to the tip of the peak (Figure 1).

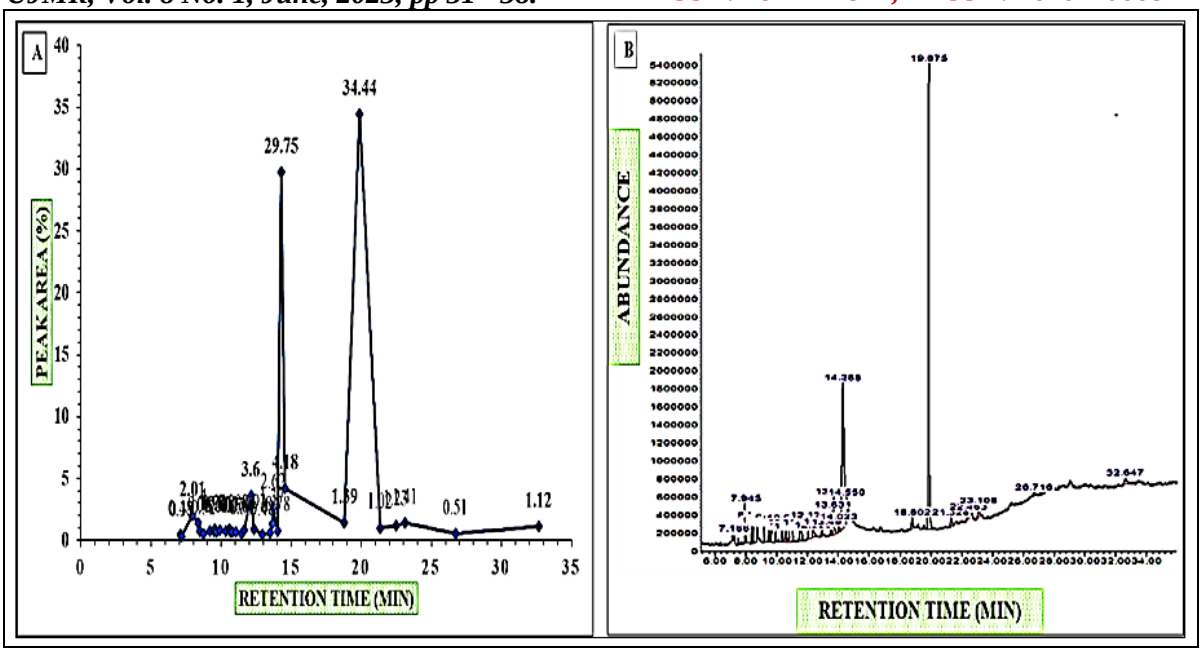

Figure 1: Chromatogram of the gas chromatography-mass spectrometry (GC/MS) analysis of the ethanol extract of *Thesium viride* showing the retention time, peak area (A), and abundance of bioactive compounds (B)

Gas chromatography-mass spectrometry (GC/MS) profiling

Figure 2 shows the identity of the bioactive compounds in the ethanol extract of *Thesium viride* at different peak areas and retention times. The total retention time for the elution of the bioactive components was 32.65 minutes. Overall, thirty-four bioactive components were identified. Bis(2-ethylhexyl) phthalate, cis-13-Octadecenoic acid, trans-13-Octadecenoic acid, n-Hexadecanoic acid, 9-Octadecenoic acid (Z), and Diethyl Phthalate were the major bioactive compounds identified, with peak areas of 34.44%, 29.75%, 4.18%, 3.60%, 2.62%, and 2.01% respectively. Others were found in trace amounts (Figure 2).

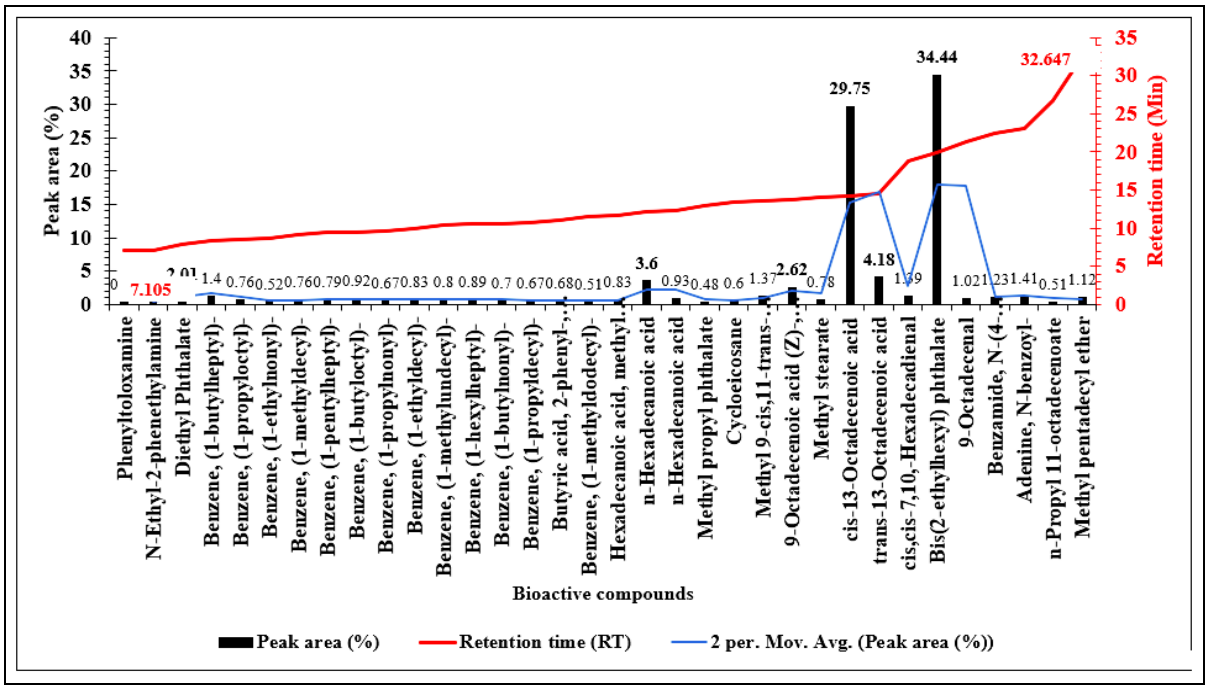

Figure 2: Gas chromatography-mass spectrometry (GC/MS) profiling of the ethanol extract of *Thesium viride* depicting the retention time, a bioactive compound identified, and the peak area

Acute oral toxicity studies of *Thesium viride* in Balb/c mice

Table 2 is the clinical and behavioural observation of the acute toxicity studies of different fractions of *Thesium viride* on Balb/c mice. All animals that were given lower and higher doses of the different fractions of the There was no mortality recorded across all the fractions. Hence, the median lethal dose was calculated to be greater than 5000 mg/kg for all the fractions (Table 2).

extracts showed mild signs of toxicity, and mild agitations, piloerections, defaecation, urination, and sleepiness characterised their behavioural responses. No signs of convulsion, diarrhoea, abdominal writhing, tremor and ptosis were observed.

Table 2: Clinical observation and behavioural response of Balb/c mice subjected to the acute toxicity studies of different fractions of whole plant extract of *Thesium viride*

Parameters	First group, 2000 mg/kg body weight					Second group, 5000 mg/kg body weight				
	Ethanol (n = 2)	N-Hexane (n = 2)	Ethyl acetate (n = 2)	N-butanol (n = 2)	Aqueous (n = 2)	Ethanol (n = 2)	N-Hexane (n = 2)	Ethyl acetate (n = 2)	N-butanol (n = 2)	Aqueous (n = 2)
Agitation	+	+	+	+	+	+	+	+	+	+
Convulsion	×	×	×	×	×	×	×	×	×	×
Piloerection	+	+	+	+	+	+	+	+	+	+
Stretch	×	×	×	×	×	×	×	×	×	×
Tachycardia	×	×	×	×	×	×	×	×	×	×
Defecation	+	+	+	+	+	+	+	+	+	+
Diarrhoea	×	×	×	×	×	×	×	×	×	×
Urination	+	+	+	+	+	+	+	+	+	+
Abdominal writhing	×	×	×	×	×	×	×	×	×	×
Tearing	×	×	×	×	×	×	×	×	×	×
Cyanosis	×	×	×	×	×	×	×	×	×	×
Sleepiness	+	+	+	+	+	+	+	+	+	+
Tremors	×	×	×	×	×	×	×	×	×	×
Ptosis	×	×	×	×	×	×	×	×	×	×
Mortality	×	×	×	×	×	×	×	×	×	×

+: Observed, ×: Not observed

DISCUSSION

The qualitative phytochemical constituents of *Thesium viride* determined using different solvent extracts show that all contain cardiac glycosides, carbohydrates, flavonoids, tannins, and saponins. Additionally, steroids, triterpenes, and alkaloids are present in all the extracts except in the ethyl acetate and aqueous fractions. In the present study, anthraquinones were absent in all the fractions. The result contrasts with the findings of Shehu *et al.* (2016), who reported the presence of anthraquinones in the chloroform and ethyl acetate fractions of *Thesium viride* (Shehu *et al.*, 2016, 2017). The presence of phytochemicals such as flavonoids, alkaloids, saponins, steroids, triterpenes, and glycosides has been reported to be valuable because of their anti-plasmodial and anti-trypanosomal efficacies and their selective mode of action (Mohammed *et al.*, 2017; Salisu *et al.*, 2017; Oluyemi *et al.*, 2020; Ungogo *et al.*, 2020; Yun *et al.*, 2021).

Furthermore, the GC-MS analysis of the concentrated ethanol extract presented many compounds with diverse uses. Thirty-four bioactive components were identified in the ethanol extract of *Thesium viride* at different peak areas and retention times. The total retention time for the elution of the bioactive components was 32.65 minutes. Retention time is the time that passes between injection and elusion of a sample component from the column, and this parameter is used to differentiate between the components. Another parameter is the peak size, which also gives information on the sample's concentration and type of components. The greater the size of the peaks, the higher the component concentration in a sample. The major bioactive compounds identified in the ethanol extract of the whole plant of *Thesium viride* were Bis(2-ethylhexyl) phthalate, cis-13-Octadecenoic acid, trans-13-Octadecenoic acid, n-Hexadecanoic acid, 9-Octadecenoic acid (Z), and Diethyl Phthalate. Although there is no available research to test the anti-protozoal activities of the above bioactive compounds, the antimicrobial, antioxidant, antiarthritic, hypocholesterolemic, and anti-inflammatory properties of octadecanoic acid and hexadecanoic acid have been reported (Salisu and Shema 2019; Salisu et al., 2019; Kamaruding *et al.*, 2020; Kumari *et al.*, 2020; Adamu et al., 2022) . *Plasmodium* and trypanosomes have been implicated in inducing oxidative stress and inflammatory responses in the host. The presence of these compounds in *Thesium viride* is a potentially

vital compound to be explored in further studies.

All animals administered lower and higher doses of ethanol and n-butanol crude extracts of *Thesium viride* showed no toxicity; their behavioural responses were normal, and no deaths were recorded. Hence, the median lethal dose was greater than 5000 mg/kg, indicating that the extract is practically non-toxic with a high safety level when administered orally in mice according to Loomis and Hayes (1996) toxicity classification. The non-toxicity of *Thesium viride* in mice concurs with the findings of Shehu *et al*. (2017) for an ethanol extract of *Thesium viride* orally administered in rats (Shehu *et al*., 2017).

CONCLUSION

Based on the results obtained, the study concludes that the phytochemical analysis of *Thesium viride* whole plant extracts revealed the presence of cardiac glycosides, carbohydrates, flavonoids, tannins, saponins, steroids, triterpenes, and alkaloids. In addition, the GC-MS analysis of the ethanol extract of *Thesium viride* revealed the presence of thirty-four bioactive compounds, the major ones being Bis(2-ethylhexyl) phthalate, cis-13-Octadecenoic acid, trans-13-Octadecenoic acid, n-Hexadecanoic acid, 9-Octadecenoic acid (Z), and Diethyl Phthalate with peak areas (concentrations) of 34.44%, 29.75%, 4.18%, 3.60%, 2.62%, and 2.01%, respectively. The median lethal dose (LD50) of *Thesium viride* whole plant extract was higher than 5000 mg/kg, indicating that the extract is practically non-toxic and orally safe when administered in mice.

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APPENDIX I: The GC/MS profile of ethanol extract of *Thesium viride* showing the retention time, bioactive compounds identified, and the peak area

Peak	Retention Time (RT)	Bioactive compound	Peak area (%)	Ref
1	7.105	Phenyltoloxamine	0.48	116338
2	7.166	N-Ethyl-2-phenethylamine	0.35	24005
3	7.945	Diethyl Phthalate	2.01	84999
4	8.365	Benzene, (1-butylheptyl)-	1.4	95150
5	8.489	Benzene, (1-propyloctyl)-	0.76	95151
6	8.738	Benzene, (1-ethylnonyl)-	0.52	95148
7	9.175	Benzene, (1-methyldecyl)-	0.76	95153
8	9.47	Benzene, (1-pentylheptyl)-	0.79	108064
9	9.525	Benzene, (1-butyloctyl)-	0.92	108058
10	9.665	Benzene, (1-propylnonyl)-	0.67	108063
11	9.913	Benzene, (1-ethyldecyl)-	0.83	108046
12	10.345	Benzene, (1-methylundecyl)-	0.8	108071
13	10.571	Benzene, (1-hexylheptyl)-	0.89	121340
14	10.648	Benzene, (1-butylnonyl)-	0.7	121335
15	10.787	Benzene, (1-propyldecyl)-	0.67	121338
16	11.041	Butyric acid, 2-phenyl-, 1,1,1-trifluoroprop-2-yl ester	0.68	120706
17	11.47	Benzene, (1-methyldodecyl)-	0.51	121345
18	11.618	Hexadecanoic acid, methyl ester	0.83	130822
19	12.124	n-Hexadecanoic acid	3.6	117419
20	12.35	n-Hexadecanoic acid	0.93	117419
21	12.949	Methyl propyl phthalate	0.48	85007
22	13.491	Cycloeicosane	0.6	140274
23	13.631	Methyl 9-cis,11-trans-octadecadienoate	1.37	153865
24	13.699	9-Octadecenoic acid (Z)-, methyl ester	2.62	155748
25	14.023	Methyl stearate	0.78	157885
26	14.285	cis-13-Octadecenoic acid	29.75	142083
27	14.55	trans-13-Octadecenoic acid	4.18	142094
28	18.802	cis, cis-7,10, -Hexadecadienal	1.39	98687
29	19.875	Bis(2-ethylhexyl) phthalate	34.44	233372
30	21.328	9-Octadecenal	1.02	126829
31	22.463	Benzamide, N-(4-methylphenyl)-Ethanone	1.23	75379
32	23.106	Adenine, N-benzoyl-	1.41	101117
33	26.716	n-Propyl 11-octadecenoate	0.51	182559
34	32.647	Methyl pentadecyl ether	1.12	104447