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Research Article

PREPARATION OF HEXANE EXTRACT OF *CORDIA OBLIQUA* AND ITS ANALYSIS BY TLC, FTIR AND GCMS

Tilak Raj, Punit Kumar *
Department of Biotechnology, University Institute of Engineering and Technology, Maharshi Dayanand University Rohtak, Haryana, India
*Corresponding Author Email: punitdariyapur@gmail.com

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ABSTRACT

Since the existence of life, all species are dependent on nature for food, shelter and other requirements. From the inception of civilizations, human beings are also dependent on nature to fulfil their requirements of food, shelter and health with the help of natural sources. Natural compounds have been recognized to perform various biological functions such as; defence against pathogens and medicinal activity etc. The plants are considered to be nature's largest laboratory that synthesizes the myriad of novel compounds and may be considered as inexhaustible source of bioactive compounds. These compounds possess chemo-diversity and broadly categorized as alkaloids, terpenoids, phenols, tannins, saponins and glycosides etc. which are reported to exhibit various biological activities. In this study the hexane extract of Cordia obliqua was prepared and analyzed by FTIR and GCMS to analyze the presence of phytochemicals. GCMS analysis revealed that this extract contains large number of phytochemicals such as 3-Hexen-2-one, n-Hexadecanoic acid, Phytol, cis-9-Hexadecenal, Glycidyl palmitate, Tetracontane, Squalene, Hexatriacontane, beta.-Amyrin, Stigmasterol, Lupeol, sitosterol etc.

Keywords: Natural products; medicinal compound; phytochemicals; Stigmasterol; lupeol

INTRODUCTION

Nobody can deny the importance of natural products in the daily life. Natural products constitute major proportion of materials used in daily life such as; food stuffs, clothes, shelter and medicine. At present natural products are reported possessing large number of medicinal properties such as; antiobesity properties[1,2,3], anticancer properties[4], antimicrobial and many other properties[5]. Thus, the importance of herbal plants at present cannot be ignored. Including this, medicinal uses of herbal plants also have been described in ancient medicinal literature such as Ayurveda. Even today, large numbers of people in developing countries are consuming herbal medicines. It is found that today herbal medicines contribute the significant fraction of presently available drugs. Herbs are sources of structural diversity of phytochemicals which exhibit chemodiversity. It is assumed that chemodiversity leads to functional diversity of phytochemicals**.** The important bioactive compounds present in plants are secondary metabolites mainly glycosides, flavonoids, alkaloids, tannins, phenolic and antioxidants. These secondary metabolites are responsible for play vital function of plants. It is widely known that the presently available drugs seem to be insufficient to meet the demand against infectious disease due to development of resistance in microorganisms against these drugs. Thus, there is an urgent need to explore novel phytochemicals that have pharmaceutical potential to combat re-emerging of infectious diseases**.** Now a day, researchers are focusing their attention towards herbal medicines mentioned in ancient medical literature to hunt for novel compounds.

Due to the climatic conditions, India is enriched in plant biodiversity and according to India's fourth national report to the convention on biological diversity, India is considered home of about 7% of world flowering plant (angiosperms). In India, about 17,527 species of flowering plants have been reported under 247 families and 2984 genera[6]. Majority of these flowering plants have been used as medicinal herbs since ancient time to cure routine health problems such as; cold, cough, healing, arthritis, fever and food poisoning etc.

Cordia obliqua (Common name-Clammy Cherry) is a medium sized deciduous dicotyledonous plant which belongs to family Boraginaceae. This family contains approximately 2700 plant species which are present around the world in subtropical, tropical and warmer regions. It is assumed that about 300 species of genus *Cordia* are present worldwide and out of them 13 are present in India[7]. It is reported that two forms of *Cordia obliqua* Willd. are present, which bears two different types of fruits as small size and slightly larger size. It was observed that plants having smaller fruits are commonly present[8]. The plant '*Cordia obliqua*' is reported to possess many medicinal properties such as antipyretic, diuretic, expectorant, purgative, anthelmintic, hepatoprotective, antimicrobial, respiratory stimulant, analgesic and anti-inflammatory compound. Moreover the fruits of this plant may be consumed directly, as pickle and as vegetables[8]. In rural areas the gum (mucilage) obtained from this plant is being used for pasting sheets of paper. Mucilage from this plant also forms sustained release material and thus the mucilage isolated from this plant may be used in tablet formulations[9].

It has been previously reported that hexane extract of leaves of *Cordia obliqua* contains different types of phytochemicals such as sterol, terpenes, and alkaloids etc[5]. Moreover, this extract exhibited remarkable antimicrobial activity against bacteria (*Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, E. coli* and *Klebsiella pneumonia*) and fungi (*Aspergillus*

niger, *Aspergillus flavus* and *Aspergillus fumigates*)[5]. On the basis of previously reported medicinal importance and antimicrobial properties of hexane extract of *Cordia obliqua,* the present study was conducted to analyze the presence of different phytochemicals in the hexane extract. The hexane extract of the leaves was prepared and processed by TLC, GCMS and FTIR to analyze the presence of phytochemicals.

MATERIALS AND METHODS

In present study all consumables (reagents, chemicals, culture medium ingredients, biological kits and raw materials) were procured from Hi-Media (India), Sisco Research Laboratory (India), Merck (India), Sigma-Aldrich (India), Tarsons (India) and Borosil (India). Glassware and plasticwares were purchased from Borosil (India), JSGW (India) and Tarsons (India). Non-conventional raw materials and other consumables were purchased from local market of Rohtak, Haryana (India). Instrumentation used in this study was as; water distillation unit (JSGW, India), Centrifuge (REMI, India), Hot air oven (Macro scientific work, India), Rotary vacuum evaporator (Hansen, Korea), Lyophilizer (Hicon, India), and FTIR (Bruker, USA).

Selection of Plant

For this research work, the medicinal plant '*Cordia obliqua*' was selected and collected from botanical garden of Maharshi Dayanand University Rohtak, Haryana (India). Base of selection of this plant was its use in the conventional system of medicines and their antimicrobial potential.

Sample processing and preparation of crude extracts of plants

The leaves of above said plant were collected from site and processed for extract preparation. The leaves of different plants were washed in laboratory with tap water for five minutes and then these were washed with double distilled water to ensure that all the dust particles were removed from leaves during washing. The washed leaves were cut into small pieces and left for drying under shade until they were able to be processed into fine powder. The dried leaves were grinded into fine powder using mortar and pestle. 100 gm of the fine powder from each plant was taken and used for extraction of bioactive metabolites. The organic solvent hexane[5] was mixed with dried powder of leaves, in the ratio of 1: 10 (w/v) and left for 24h in shaker. The organic suspensions were left for settle down of leaves powder and supernatant was filtered thrice, through a Whatman no. 1 filter paper. The solvent was further evaporated in rotary vacuum evaporator at 40^{o}C until dried extract was obtained. This extract was referred as the crude extract and stored for further processing. The extract was dissolved in 5 ml of hexane to be used for chromatographic analysis.

Thin Layer Chromatography (TLC) of plant extract

TLC plates (aluminium-backed silica plates; Merck 60 F254, Germany) of 10 X 5 cm size were taken and a line using pencil was drawn at 1.5 cm above from base of plate and spots were made to load sample. About 1.0 cm of height of organic solvent (hexane and ethyl acetate, 80:20v/v) was poured into clean and dry glass chamber. This chamber was covered with air tight lid or aluminium foil and was left for 30min so that it was soaked with vapours of organic solvent. 20µl of plant extract solution was transferred at each spot on TLC plate and the samples were dried using hair dryer at room temperature. TLC plate was kept in mobile phase and allowed to run the column upto 9cm. The different compounds through TLC are detected on the basis of retardation factor (Rf) value. The retardation factor was calculated according to following formula.

$$\mathrm{Rf} = \frac{\text{distance travelled by solute}}{\text{distance travelled by solvent}}$$

Fourier Transform Infrared Spectroscopy (FTIR) and Gas chromatography mass spectroscopy (GCMS) of the plant extract

FTIR analysis of crude extract was done in the department of Biotechnology, U.I.E.T., Maharshi Dayanand University Rohtak, Haryana, India. FTIR analysis was performed by using Attenuated Total Reflectance (ATR) method in which approximately 50µl of sample was loaded to FTIR analyzer and FTIR analysis was conducted using OPUS software.

GCMS study was conducted at Advance Instrumentation Research Facility (AIRF), JNU New Delhi, India. For GCMS study, 2ml of plant extract was filtered by 0.20µm syringe filter and provided for further analysis.

RESULTS AND DISCUSSION

In this study the hexane extract of plant leaves was prepared in hexane. The isolated extract was further analyzed for the presence of phytochemicals through TLC, FTIR and GCMS.

Isolation of extract from leaves of *Cordia obliqua*

The yield of crude extract in hexane was 0.58% (w/w). This crude extract was further analyzed. It is well known chemistry fact that 'like dissolve like' thus the type of crude extract prepared in respective solvents exhibited the solubility of phytochemicals in particular solvents. Moreover, the amount of the phytochemicals present in plant extract depends on the geographical location and climate of the plant in which plants are grown, age and health of plant and types of leaves used for extract preparation. Including this procedural loss also affect the isolation of phytochemicals in plant extract.

TLC analysis of the hexane extract

It is evident that chromatography is one of the best suited method to fractionize the compounds from the isolated crude extract. It is quick, simple and inexpensive chromatographic technique which is based on capillary action. It provides the information about the presence of several compounds in a plant extracts by comparing Rf (Retardation factor) of a compound with respect to the Rf of known compound. In addition spraying of phytochemical screening agents can be done on TLC plate after completion of chromatography, which causes development of color according to the phytochemicals present in the plant extract[10]. In this study different bands of extract were obtained representing different phytochemicals present in extract (Figure 1). These bands represented different group of phytochemicals and different Rf values. It was found that in this study the obtained Rf values were 0.133, 0.266, 0.44, 0.75 etc. Though, TLC enables the detection of phytochemical using Rf value of standard (pure) compound and test but this TLC chromatogram reveled presence of large number of phytochemicals present in the crude extract. Further the TLC profile of the crude extract would be different in different mobile phases.

FTIR analysis of hexane extract

Fourier transform infrared (FTIR) spectroscopy is technique used to provide absorption or emission of infra red (IR) spectrum of

sample by non-destructive method. In this technology, Fourier transform is used to convert IR raw data into spectrum. The interferogram obtained by FTIR provides reciprocal length dimension (L^{-1}), which is the dimension of the wave number. The spectral resolution in cm^{-1} is equal to the reciprocal of the maximal retardation in cm. In this study, hexane extract of *Cordia obliqua* was analyzed by FTIR and different peaks were obtained with different wave numbers. These peaks represented different functional groups and possibility of different compounds present in investigated extract. Including this, library of the compounds was also checked by spectrum search tool of OPUS software (Bruker). The peaks were analyzed by online available peak table[11,12] and represented different functional groups (Table 1, Figure 2). The library search of this FTIR spectrum was also used which revealed the presence of different compounds in the crude extract (Figure 3).

GCMS analysis of hexane extract

GCMS enables the analysis of different volatile compounds present in sample. This chromatographic approach causes separation of molecules on the basis of polarity and interaction between stationary phase and carrier gas. Mass spectroscopy provides the analysis of mass. The analysis of the library of different compounds provides the information about type of phytochemical present in crude extract, its retention time and peak area. In this study, the hexane extract of Cordia obliqua was analyzed by GCMS and different peaks with respect to different retention time were obtained which exhibited different compounds present in the crude extract (Table 2). These different compound were separated on the basis of different m/z ratios. The analysis of hexane extract by GCMS revealed that variety of phytochemicals were obtained as ethanone, 1-(3-ethyloxiranyl) at peak number 2,4,6 and 7 with retention time (R. Time) and peak area 4.716, 5.029, 5.330 ,5.504 min and peak area 10.30 %, 6.39 %, 1.42 %, and 1.02 % respectively. 3-Hexen-2-one was obtained at retention time 5.854min with peak area of 6.78%. Similarly stigmasterol and gamma-sitosterol were reported at peak number 62 and 65 with retention time 35.065 and 35.937 min and having peak area 3.55 % and 9.24 % respectively. The peak number 67 represent the beta-Amyrin having retention time at 36.532 min with peak area 3.26 %. The peak number 70 denoted the presence of Lupeol having retention time 37.222 min and peak area 5.27 %. More detailed information of all peaks obtained with their retention time and peak area is given below.

The GCMS study revealed that hexane extract was containing large number (82) of phytochemicals exhibiting large number of biological activities. Such as; Lupeol (pentacyclic triterpene, figure 4), shows broad spectrum of medicinal activities in treatment of heart diseases, diabetes, cancer, renal toxicity, inflammation, arthritis and hepatic toxicity etc[13,14]. Stigmasterol (figure 4) has been found associated with cytotoxic activity, antitumor activity, antioxidant, antimutagenic, anti-inflammatory activity etc[15]. Out of the isolated compounds by GCMS, the compounds such as; lupeol, Stigmasterol and gamma-sitosterol are phytosterols. Scientific Literature supports that lupeol,[16,17] stigmasterol[18,19] and gamma-sitosterol[20] possess antimicrobial activity. Thus, it may be assumed that due to presence of these phytochemicals, hexane extract of Cordia obliqua represented antimicrobial property against different microorganisms.

FUTURE PERSPECTIVES

It was preliminary study in order to analyze the presence of different phytochemicals in hexane extract exhibiting different medicinal properties. Further study requires the isolation and purification of different phytochemicals and analysis of their antimicrobial potential. The positive outcomes of such study will possibly lead to obtain the potent bioactive compound exhibiting broad spectrum antimicrobial activities. Though, there may be synergistic effect behind different medicinal properties, thus, such possibility also needs to be ruled out by analyzing the activities of mixture of different compounds in different combinations.

CONCLUSION

Natural products are important source for medicinal compounds. These phytochemicals are classified into various groups on the basis of chemical structure and function such as phenols, flavonoids, alkaloids, tannins, saponins and glycosides etc. these compound exhibit various properties like; antimicrobial, antioxidant, anti cancerous etc. In this study, hexane extract of *Cordia obliqua* was analyzed by TLC, FTIR and GCMS. The GCMS study revealed that this extract contains large number of phytochemicals with different retention time and peak area.

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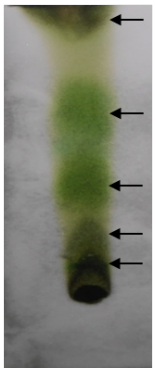

Figure 1: Analysis of hexane extract by TLC

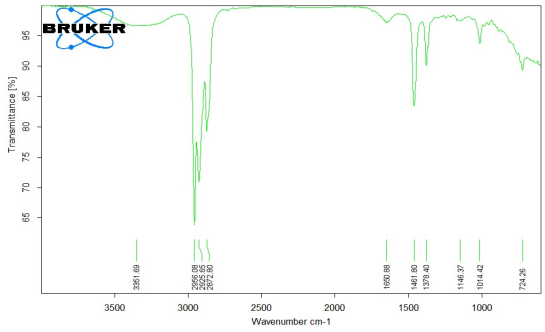

Figure 2: FTIR spectrum of hexane extract of *Cordia obliqua*

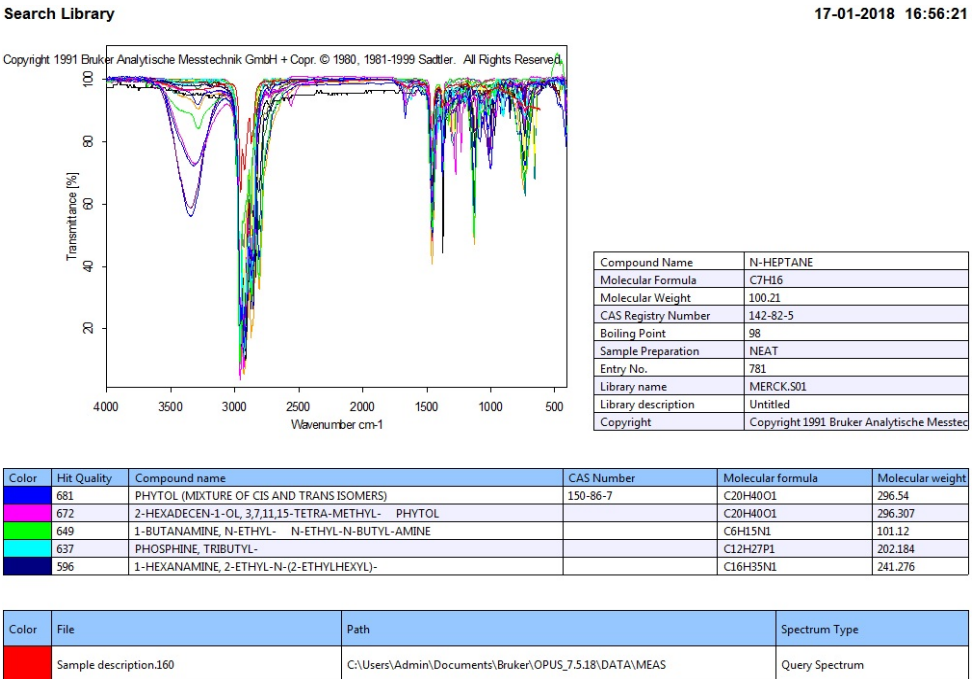

Figure 3: Library search of FTIR spectrum obtained from hexane extract

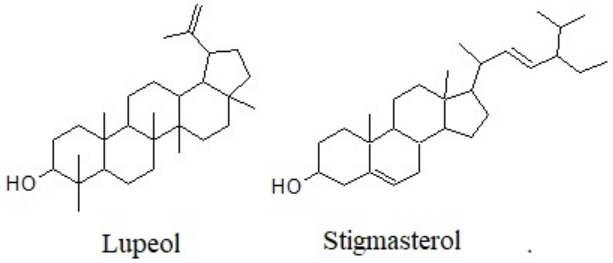

Figure 4: Structure of lupeol and stigmasterol

Table 1: FTIR analysis of hexane extract of *Cordia obliqua*

S. No.	Wave number (cm^{-1})	Functional group
1.	724.26	C-Br (Aromatic C-H Bending)
2.	1014.42	C-F
3.	1146.37	C-OH stretch
4.	1378.40	NO_2 stretch
5.	1461.80	CH_3 bend
6.	1650.88	C=C alkene, C=O amide
7.	2872.80	-C-H stretch, Alkyl C-H Stretch, -C-H aldehydic, carboxylic acid OH stretch
8.	2925.65	-C-H stretch, carboxylic acid OH stretch
9.	2956.08	-C-H stretch, carboxylic acid OH stretch
10.	3351.69	water OH Stretch, phenol/alcohol OH stretch, carboxylic acid OH stretch, N-H stretch

Table 2: Detailed of chromatogram obtained by GCMS of hexane extract

Peak#	R. Time	Area	Area%	Name
1	4.319	1421617	0.18	1-Butene, 2,3,3-trimethyl-
2	4.716	82441940	10.30	ETHANONE, 1-(3-ETHYLOXIRANYL)-
3	4.848	2368083	0.30	Pentane, 3-ethyl-2,4-dimethyl-
4	5.029	51104774	6.39	ETHANONE, 1-(3-ETHYLOXIRANYL)-
5	5.215	2764175	0.35	Pentane, 3-ethyl-3-methyl-
6	5.330	11364366	1.42	ETHANONE, 1-(3-ETHYLOXIRANYL)-
7	5.504	8187332	1.02	ETHANONE, 1-(3-ETHYLOXIRANYL)-
8	5.667	2026728	0.25	Propanoic acid, 1-methylethyl ester
9	5.854	54206302	6.78	3-Hexen-2-one
10	6.260	2996967	0.37	Cyclopropane, 1,1,2,2-tetramethyl-
11	6.312	2422890	0.30	Cyclopropane, 1,1,2,2-tetramethyl-
12	6.452	4411343	0.55	HEPTANE, 3-(CHLOROMETHYL)-
13	6.564	1318049	0.16	2-Pentanone, 3,3,4,4-tetramethyl-

14	6.888	20003967	2.50	2-Propanol, 1,1'-oxybis-
15	7.253	9287626	1.16	1-Propanol, 2-(2-hydroxypropoxy)-
16	7.362	4358081	0.54	1-Propanol, 2-(2-hydroxypropoxy)-
17	7.799	1328941	0.17	7-Octen-2-ol, 2,6-dimethyl-
18	7.862	1560706	0.20	BIS(1-METHYL-2-HYDROXYETHYL)ETHER
19	8.525	522809	0.07	Hexahydro-pentalene-1,6-dione
20	15.048	1769761	0.22	3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-
21	15.875	1336623	0.17	2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl-,
22	18.270	569299	0.07	Eicosane
23	18.651	931971	0.12	1-Dodecanol, 3,7,11-trimethyl-
24	20.116	4577044	0.57	Neophytadiene
25	20.175	2754575	0.34	2-Pentadecanone, 6,10,14-trimethyl-
26	20.414	2687734	0.34	Phthalic acid, butyl undecyl ester
27	20.667	2036723	0.25	2-HEXADECEN-1-OL,3,7,11,15-TETRAMETHYL-,[R-[R
28	21.750	16275277	2.03	n-Hexadecanoic acid
29	22.674	4916784	0.61	Palmitic Acid, TMS derivative
30	23.490	23151649	2.89	Phytol
31	23.834	18386152	2.30	cis-9-Hexadecenal
32	24.077	2323098	0.29	Octadecanoic acid
33	24.139	2578083	0.32	Phytol, TMS derivative
34	24.625	963813	0.12	5,8,11-Eicosatrienoic acid, (Z)-, TMS derivative
35	25.508	8011850	1.00	Glycidyl palmitate
36	26.099	7528063	0.94	4,8,12,16-Tetramethylheptadecan-4-olide
37	26.673	1163379	0.15	Tetracontane
38	26.988	691439	0.09	1,8,11,14-Heptadecatetraene, (Z,Z,Z)-
39	27.554	9346946	1.17	1,8,11-Heptadecatriene, (Z,Z)-
40	27.662	14190677	1.77	ETHYL (9Z,12Z)-9,12-OCTADECADIENOATE #
41	27.857	1628818	0.20	PREGNANE, SILANE DERIV.
42	27.989	4701166	0.59	Tetracontane
43	28.382	2701191	0.34	Bis(2-ethylhexyl) phthalate
44	28.725	1985959	0.25	HEXADECANOIC ACID, 4-[(TRIMETHYLSILYL)OXY]B
45	29.153	1982980	0.25	Tetracontane
46	29.664	1062972	0.13	2-Propenoic acid, 3-phenyl-, 1-ethenyl-1,5-dimethyl-4-hexen
47	30.089	5429570	0.68	Tetracontane
48	30.875	2463888	0.31	Tetracontane
49	30.979	11512501	1.44	Squalene
50	31.148	5897389	0.74	Henicosanal
51	31.608	37578362	4.70	HEXATRIACONTANE
52	32.356	6645580	0.83	Tetracontane
53	32.439	4625066	0.58	Octacosyl acetate
54	32.716	6876625	0.86	Henicosanal
55	32.849	1775764	0.22	.gamma.-Tocopherol
56	33.242	28355486	3.54	Hexatriacontane
57	33.330	16541857	2.07	1-Hexacosanol
58	33.567	5168852	0.65	2,5,7,8-TETRAMETHYL-2-(4,8,12-TRIMETHYLTRIDEC
59	34.220	3457975	0.43	HEXATRIACONTANE
60	34.353	8786412	1.10	Octacosyl acetate
61	34.777	10039272	1.25	Henicosanal
62	35.065	28428820	3.55	Stigmasterol
63	35.395	4304457	0.54	TETRAPENTACONTANE
64	35.702	3243696	0.41	1-EICOSANOL
65	35.937	73921231	9.24	.gamma.-Sitosterol
66	36.427	11705198	1.46	Stigmasta-5,24(28)-dien-3-ol, (3.beta.,24Z)-
67	36.532	26117347	3.26	.beta.-Amyrin
68	36.925	1657490	0.21	Octacosyl acetate
69	37.027	1321097	0.17	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-
70	37.222	42139240	5.27	Lupeol
71	37.540	11964048	1.50	2-HEPTADECYLOXIRANE
72	37.658	3297249	0.41	.gamma.-Sitostenone
73	38.881	2774417	0.35	2-NONADECANONE
74	39.319	2818386	0.35	Phytyl palmitate
75	39.744	4654600	0.58	3,5,7-TRIS(TRIMETHYLSILOXY)-2-[3,4-DI(TRIMETHY
76	40.278	3400198	0.43	2,6,10,14,18,22,26,30-Dotriacontaoctaen-1-ol, 3,7,11,15,19,
77	40.662	3235089	0.40	Octacosyl acetate
78	41.587	3280429	0.41	2-HEPTADECYLOXIRANE
79	43.581	2199990	0.27	CYCLOPROPANEOCTANOIC ACID, 2-OCTYL-,METHY
80	43.922	2129439	0.27	(9Z,12Z,15Z)-(E)-3,7-Dimethylocta-2,6-dien-1-yl octadeca-9
81	44.392	4721032	0.59	SOLANESOL
82	45.398	3222325	0.40	Squalene
		800041099	100.00	

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