ISOLATION AND IDENTIFICATION OF BIOACTIVE COMPOUNDS IN CHLOROFORM EXTRACT OF *PHYLA NODIFLORA* (L) GREENE.

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Abstract: The plants which have an array of substances, produce several bioactive compounds which help in curing diseases, relieving pain and maintaining good health are termed as medicinal plants. They are recognized in the pharmaceutical industry for the development of new drugs for human benefit and chemotherapeutics as herbal remedies. Hence, all the parts of the plants have been used from the ancient times as folklore or traditional medicine especially in India, China, etc.

Phyla nodiflora (L) Greene, is one such plant which is in common use since ancient. The different extracts are used for curing fever, cold, diarrhoea, ulcers, pain in knee joints, asthma, bronchitis, as anti-inflammatory, gonorrhea, hair affliction, dandruff etc as it contain a variety of constituents such as sugar, triterpenoids, flavonoids, phenol, steroids, essential oils, resins, tannins and many others, and the present study aimed to screen and identify the novel compounds present in *P. nodiflora* using HPLC-MS and GC-MS with different organic solvents. Of which, 26 bioactive compounds were identified in the chloroform extract, which are not reported elsewhere.

Keywords: *Phyla nodiflora*, HPLC-MS and GC-MS, Phenol,3,5-bis(1,1-dimethylethy), 1,2- Benzenedicarboxylic acid, bis, Dibutyl phthalate.

I INTRODUCTION

In today's health care system, nearly 80% of the total global population, depend only the traditional medicine according to Maher [1], which is a steep increase from 60% as per Kumar [2]. This is due to validation of bioactive compounds, and develop into drug forms through frontier science. However, only 13,000 plants have been studied [3]. But many of them need to be explored further.

One such plant is *Phyla nodiflora* commonly known as *Lippia*, belonging to Verbenaceae. It is aquatic/terrestrial (marshy), evergreen, fast -growing, mat-forming and prostrate perennial plant, distributed in India [4].

This plant is common in use since ancient. The different extracts are used for curing fever, cold, diarrhea, ulcers, pain in knee joints, asthma, bronchitis, as anti-inflammatory, gonorrhea, hair affliction, dandruff etc [5] as it contain a variety of constituents such as sugar, triterpenoids, flavonoids, phenol, steroids, essential oils, resins, tannins and many others [6]. According to Agarwal [7] the aerial parts are used as anodyne, antibacterial, diuretic, parasiticide, refrigerant, febrifuge and cooling. *P. nodiflora* has plenty of bioactive compounds in methanolic extracts (8 compounds) by Sudha Srinivasan [8], and the present study aimed to screen and identify the novel compounds present in *P. nodiflora* using HPLC-MS-MS and GC-MS with different organic solvents.

II MATERIALS AND METHODS

2.1 Plant material- Sources and Extraction

Leaves of *Phyla nodiflora* was collected from Kanapathikurichi in Cuddalore District, Tamil Nadu.

Leaves of *Phyla nodiflora* was shade dried and powdered. Twenty grams of powdered leaves was extracted successively using solvents *viz*., petroleum ether, chloroform, acetone, ethanol and methanol. In each solvent, the plant material was soaked for 24hrs at 30±2°C, filtered. All the filtrates were pooled and evaporated under vacuum in a rotary evaporator at 190rpm/min [9].

2.2 Initial screening of phytoconstituents using TLC Analysis

The crude extract of all the samples were spotted in silica gel coated TLC plate (60 F254) and kept in a coupling jar with lid containing its respective solvent to wet the bottom of the plate without submerging in the spots and allowed to run. R_f value was calculated.

2.3 Fractionation of plant material using column chromatography

Crude concentrate (30g) of the chloroform extract of *Phyla nodiflora* was chromatographed on silica gel (SiO_2 100-200 mesh size) column using sequentially ethanol: chloroform as effluent and different coloured fractions were collected.

The fractioned samples were subjected to TLC glass plate to separate and isolate the number of compounds as mentioned earlier and observed under UV light. The prominent spots were scraped and dissolved in the ethanol solvent to separate the active compounds using GC-MS [10].

2.4 HPLC-MS

Chromatographic analysis was performed using an ELITE LaChrom high- performance liquid chromatography (HPLC-MS) system coupled with a L-2420 UV-Vis detector, a L-2200 autosampler, and a L-2130 pump. Chromatographic data were processed by Hitachi Model D-2000 Elite chromatography data station software. The gradient elution program was conducted as follows: a gradual increase of Retention Time from 0.100, 4.953 was followed. The wavelength of the detector was set at 250 nm and the sample injection volume was 20 μl.

2.5 Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The spectra were recorded for its molecular configuration using spectroscopic analysis and the data are interpreted.

The GC-MS analysis was performed on a combined GC-MS instrument (ITQ 900 Model of Thermo Fisher Scientific make) using a HP-5 fused silica gel capillary column. The method to perform the analysis was designed for both GC and MS. 1 µL aliquot of sample was injected into the column using a PTV injector whose temperature was set at 275ºC. The GC program was initiated by a column temperature set at 60ºC for 5 min, increased to 300ºC at a rate of 8 C/min, held for 10 min. Helium was used as the carrier gas (1.5 mL/min). The mass spectrometer was operated in EI mode with mass source was set at 200ºC. The chromatogram and spectrum of the peaks were visualized. The particular compounds present in the samples were identified by matching their mass spectral fragmentation patterns of the respective peaks in the chromatogram with those stored in the National Institute of Standards and Technology Mass Spectral Database library [11].

Phyla nodiflora - Habit

2.6 Identification of Compounds

Identification was based on the molecular structure, molecular moss and calculated fragments. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology [NIST]. The name, molecular weight and structure of the components of the test materials were ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The spectrum of the unknown component was compared with the spectrum of the component stored in the NIST library [12]. The crude leaf extract of *P. nodiflora* subjected to different solvents (petroleum ether, chloroform, acetone, ethanol and methanol), was screened through TLC and the R_f value was calculated. The chloroform extract of *Phyla nodiflora* which had more number of bands were purified through column chromatography and further subjected to HPLC-MS and the elute was concentrated using rotary evaporator and subjected to GC-MS analysis.

III RESULT AND DISCUSSION

3.1 Screening of active compounds

The data presented in the Table 1 and Fig.1 denoted there are many active compounds at different levels. The compounds present is varied according to the solvent used.

This data denoted the proof of many compounds in *Phyla nodiflora* with different extracts used. The extract of chloroform showed 5 bands at R_f 0.04, 0.13, 0.19, 0.24, and 0.64. Whereas, the samples extracted with petroleum ether, methanol, ethanol, acetone had only one band each at R_f 0.6, 0.54, 0.7, 0.84 respectively. This indicates that the chloroform extract had many active compounds hence, used for further study.

Table 1: TLC Analysis of *Phyla nodiflora*

Solvent	Rf Value
Methanol	0.54
Ethanol	0.70
Acetone	0.84
Chloroform	0.04, 0.13, 0.19, 0.24, 0.64
Petroleum ether	0.6

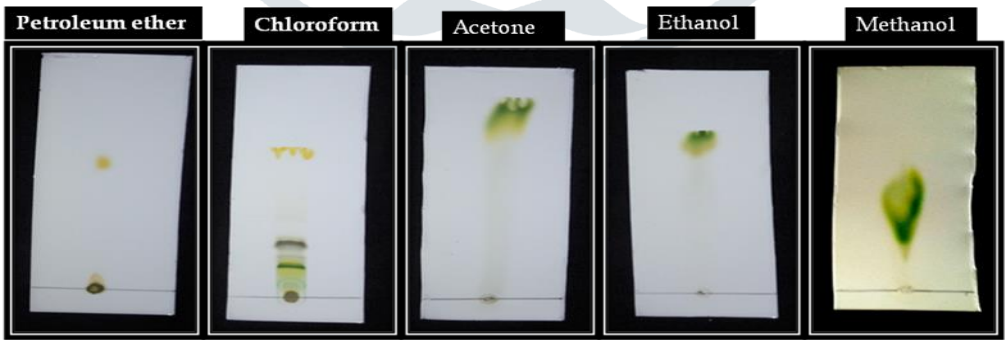
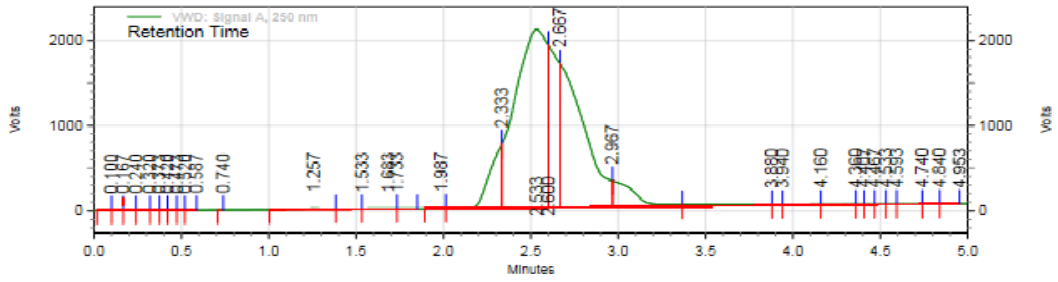


Fig.1: TLC separation of phytoconstituents of *Phyla nodiflora*

3.2 HPLC-MS-MS Analysis

Area % Report

Data File: D:\EZ ChromCompact SW\Project\Result\SYSTEM.rslt\P.nodiflora.2018-02-13 11-18-36 (GMT +05-30)SYSTEM.rslt\P.nodiflora.SYSTEM118.dat
Method: D:\EZ ChromCompact SW\Project\Method\MDL Water.met
Acquired: 2/13/2018 11:19:05 AM (GMT +05:30)
Printed: 2/13/2018 11:28:08 AM (GMT +05:30)



VWD: Signal A, 250 nm Results

Retention Time	Area	Area %	Height	Height %
0.100	2029	0.00	720	0.00
0.167	5526	0.00	1883	0.00
0.240	11478	0.00	2466	0.00
0.320	10083	0.00	1981	0.00
0.373	6071	0.00	1715	0.00
0.420	4366	0.00	1366	0.00
0.473	3593	0.00	798	0.00
0.520	1453	0.00	294	0.00
0.587	393	0.00	0	0.00
0.740	19	0.00	0	0.00
1.257	914859	0.10	123951	0.11
1.533	354744	0.04	42539	0.04
1.683	510081	0.05	41792	0.04
1.733	112669	0.01	31291	0.03
1.987	190813	0.02	38610	0.03
2.333	56954981	6.02	12602906	11.00
2.533	432252639	45.72	35117627	30.65
2.600	120409309	12.73	31971518	27.91
2.667	273528661	28.93	28262607	24.67
2.967	44455520	4.70	5154681	4.50
3.880	8628701	0.91	220994	0.19
3.940	771423	0.08	207627	0.18
4.160	2435374	0.26	162689	0.14
4.360	1718323	0.18	123233	0.11
4.407	331806	0.04	113784	0.10
4.467	387852	0.04	101698	0.09
4.533	380033	0.04	88285	0.08
4.593	295950	0.03	76104	0.07
4.740	536627	0.06	45281	0.04
4.840	205992	0.02	23605	0.02
4.953	79889	0.01	0	0.00

Totals	945501257	100.00	114562045	100.00

HPLC-MS-MS is a relatively simple and reliable technique and is ideal for the rapid comparative study of plant samples. This method is an excellent technique for quality control of drug analysis [13]. This technique provides reliable separation of substances even with closed structures. The approach of HPLC-MS-MS is more popular in the complex extracts due to its better compatibility and precision of finding the compounds [14].

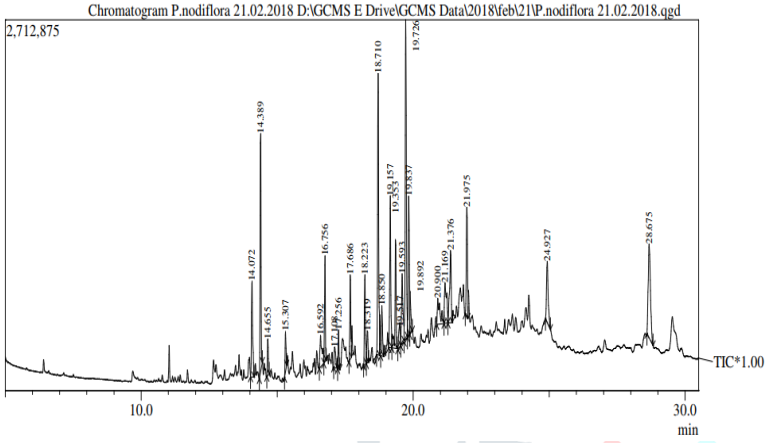
3.3 GC-MS analysis of *Phyla nodiflora*

The chloroform extract of *Phyla nodiflora* was further subjected to GC-MS analysis and the results were depicted in the following Figs. The GC-MS analysis showed 26 compounds at different RT.

Sample Information

Analyzed by	: Admin
Analyzed	: 2/8/2018 10:08:05 AM
Sample Type	: Unknown
Level #	: 1
Sample Name	: P.nodiflora 21.02.2018
Sample ID	:
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 1
Injection Volume	: 1
Data File	: D:\GCMS E Drive\GCMS Data\2018\feb\21\P.nodiflora 21.02.2018.qgd
Org Data File	: D:\GCMS E Drive\GCMS Data\2018\feb\21\P.nodiflora 21.02.2018.qgd
Method File	: D:\GCMS E Drive\GCMS Data\2018\feb\21\S.Sowmiya,Holy cross college 21.02.2018.qgm
Org Method File	: D:\GCMS E Drive\GCMS Data\2018\feb\21\S.Sowmiya,Holy cross college 21.02.2018.qgm
Report File	:

Chromatogram P.nodiflora 21.02.2018 D:\GCMS E Drive\GCMS Data\2018\feb\21\P.nodiflora 21.02.2018.qgd



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area%	Height%	A/H	Mark	Name
1	14.072	14.017	14.150	3.77	3.99	2.87	V	Eicosane
2	14.389	14.342	14.450	7.48	9.92	2.29		Phenol, 3,5-bis(1,1-dimethylethyl)-
3	14.655	14.617	14.708	1.17	1.51	2.34		OCTADECANE
4	15.307	15.267	15.350	1.31	1.66	2.40		1-Hexadecanol
5	16.592	16.550	16.717	2.31	1.33	5.29	V	Pentadecane
6	16.756	16.717	16.800	3.11	4.43	2.14	V	Hexacosane
7	17.108	17.075	17.225	1.31	0.82	4.84		2-Isopropyl-5-methyl-1-heptanol
8	17.256	17.225	17.300	1.14	1.64	2.11	V	Pentadecane, 8-hexyl-
9	17.686	17.642	17.725	2.82	3.51	2.44		n-Heptadecanol-1
10	18.223	18.175	18.275	3.20	3.74	2.60		2,6,10-TRIMETHYL,14-ETHYLENE-14-PE
11	18.319	18.275	18.367	1.32	1.34	3.00	V	2-methyltetracosane
12	18.710	18.650	18.758	10.19	11.75	2.64		1,2-BENZENEDICARBOXYLIC ACID, BIS
13	18.850	18.808	18.917	1.83	2.09	2.65	V	Heneicosane
14	19.157	19.100	19.217	5.79	6.34	2.77		TETRATRIACONTANE
15	19.353	19.217	19.408	4.18	4.54	2.80	V	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien
16	19.517	19.433	19.542	1.28	0.79	4.91		2-methylhexacosane
17	19.593	19.542	19.667	3.69	2.92	3.84	V	DOCOSANE
18	19.726	19.667	19.792	12.68	13.32	2.89	V	Dibutyl phthalate
19	19.837	19.792	19.875	5.16	5.82	2.69	V	1-Heneicosanol
20	19.892	19.875	19.967	1.39	1.72	2.44	V	HEXADECANE
21	20.900	20.867	21.133	2.48	1.09	6.94		Tetracosane
22	21.169	21.133	21.275	3.06	1.71	5.44	V	2-HEXADECEN-1-OL, 3,7,11,15-TETRAME
23	21.376	21.275	21.433	3.94	3.02	3.97	V	Dotriacontane
24	21.975	21.917	22.017	4.44	4.56	2.96		Octacosanol
25	24.927	24.867	25.042	3.53	2.55	4.21		Docosyl heptafluorobutyrate
26	28.675	28.592	28.817	7.42	3.92	5.75		Diisooctyl phthalate
				100.00	100.00			

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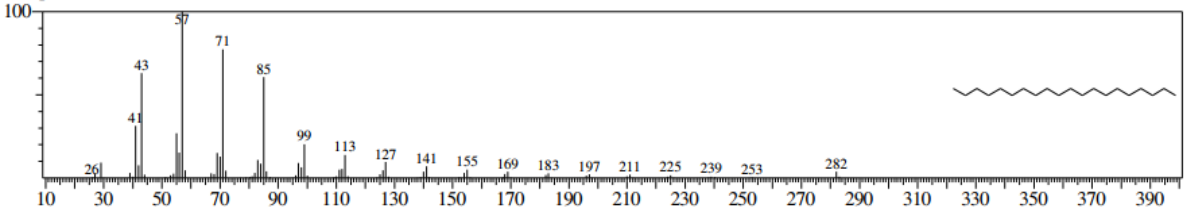
CompName:Eicosane $$ n-Eicosane $$ Icosane # $$ n-Icosane $$



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SI:94 Formula:C14H22O CAS:1138-52-9 MolWeight:206 RetIndex:1555
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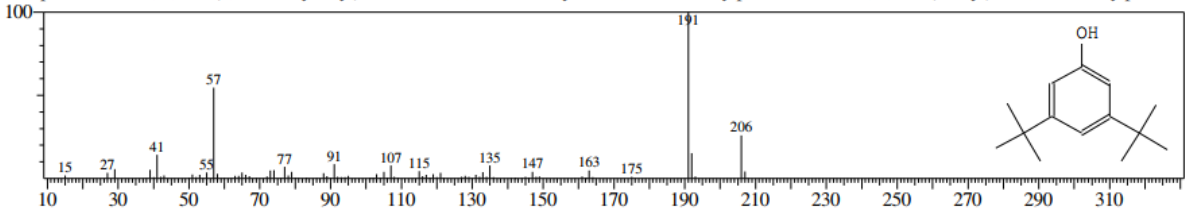
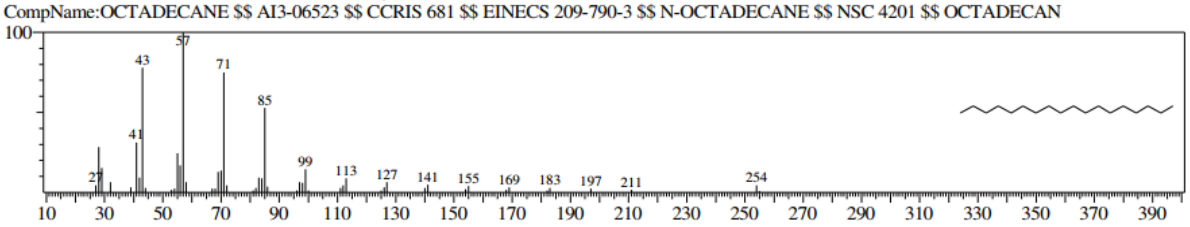


Hit#:4 Entry:180652 Library:WILEY8.LIB
SI:92 Formula:C18H38 CAS:593-45-3 MolWeight:254 RetIndex:0
CompName:OCTADECANE $$ AI3-06523 $$ CCRIS 681 $$ EINECS 209-790-3 $$ N-OCTADECANE $$ NSC 4201 $$ OCTADECAN



Hit#:1 Entry:22213 Library:NIST11s.lib
SI:95 Formula:C16H34O CAS:36653-82-4 MolWeight:242 RetIndex:1854
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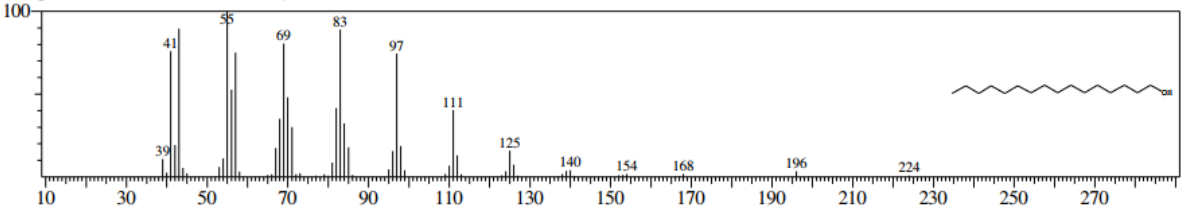

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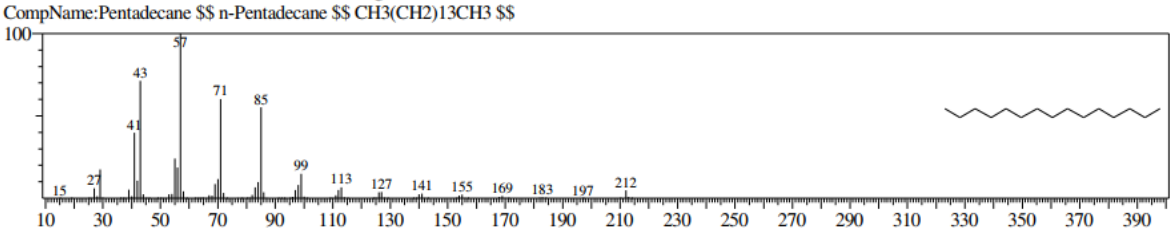

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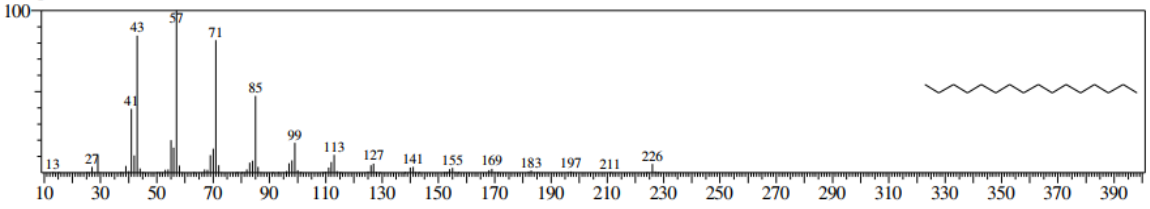

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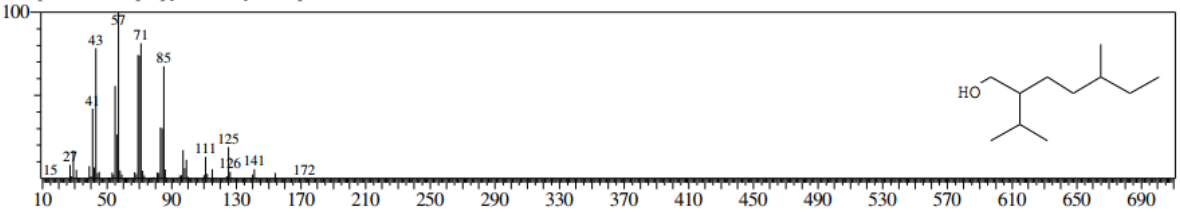

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SI:92 Formula:C21H44 CAS:13475-75-7 MolWeight:296 RetIndex:2045
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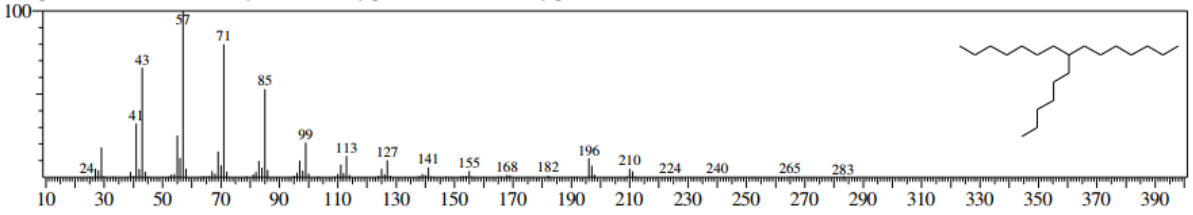

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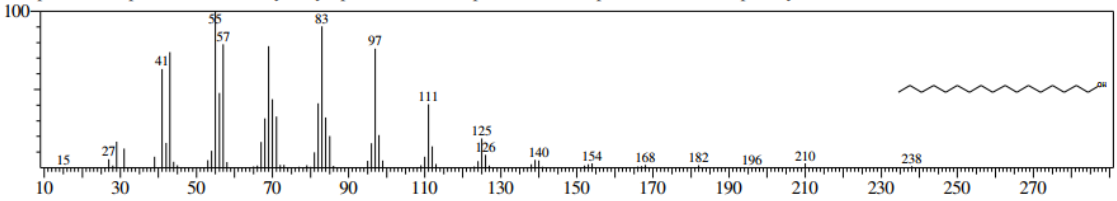

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SI:91 Formula:C20H38 CAS:504-96-1 MolWeight:278 RetIndex:0
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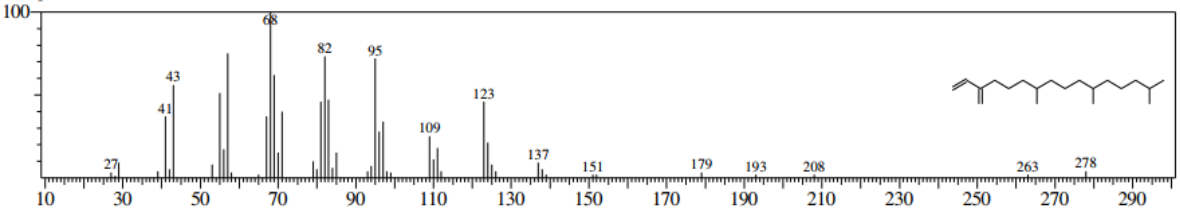

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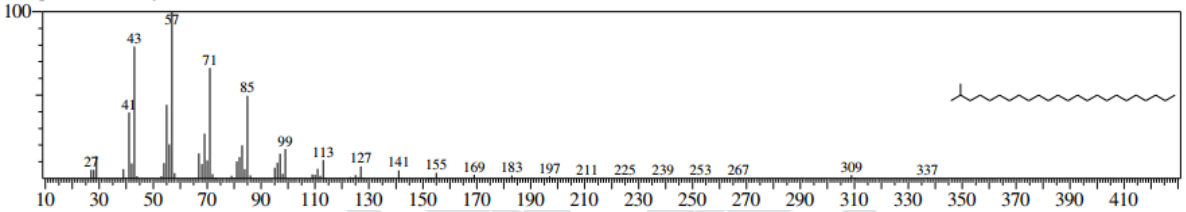
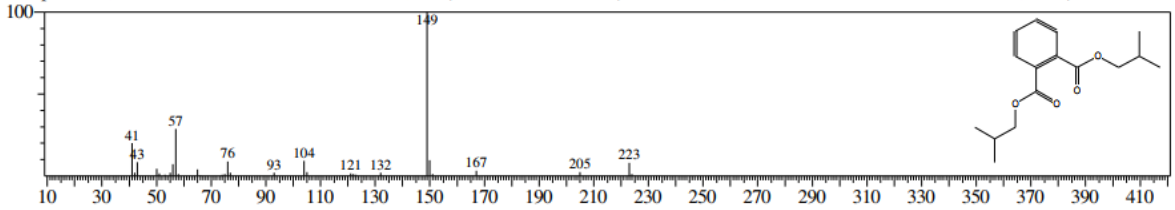
SI:87 Formula:C25H52 CAS:0-00-0 MolWeight:352 RetIndex:2442
CompName:2-methyltetracosane



Hit#:1 Entry:211760 Library:WILEY8.LIB
SI:92 Formula:C16H22O4 CAS:84-69-5 MolWeight:278 RetIndex:0
CompName:1,2-BENZENEDICARBOXYLIC ACID, BIS(2-METHYLPROPYL) ESTER $$ 1,2-BENZENEDICARBOXYLIC ACID, BIS (2-METHYLF



Hit#:5 Entry:25951 Library:NIST11s.lib
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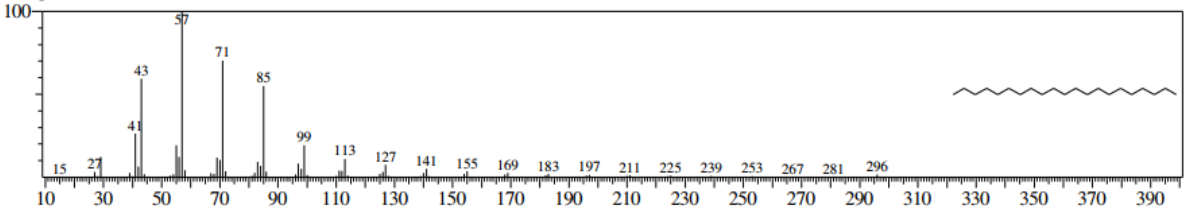

Hit#:20 Entry:370575 Library:WILEY8.LIB
SI:84 Formula:C34H70 CAS:14167-59-0 MolWeight:478 RetIndex:0
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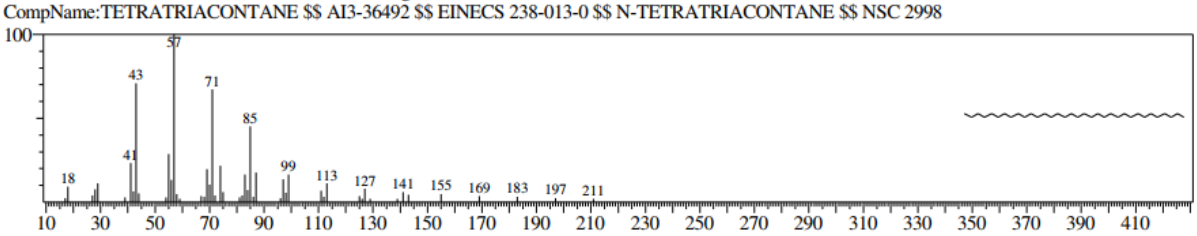
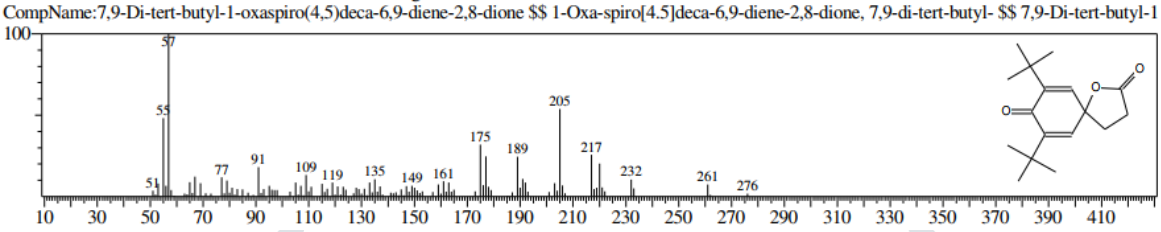
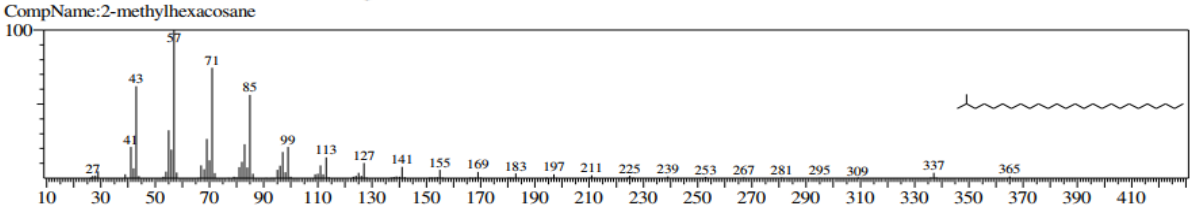


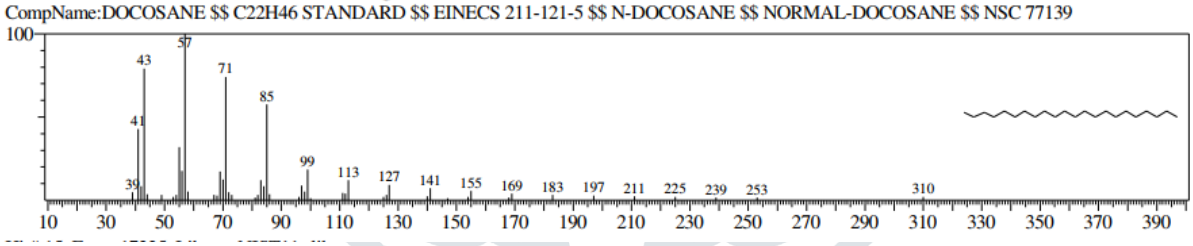
Hit#:1 Entry:99825 Library:NIST11.lib
SI:82 Formula:C17H24O3 CAS:82304-66-3 MolWeight:276 RetIndex:2081
CompName:7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione $$ 1-Oxa-spiro[4.5]deca-6,9-diene-2,8-dione, 7,9-di-tert-butyl- $$ 7,9-Di-tert-butyl-1



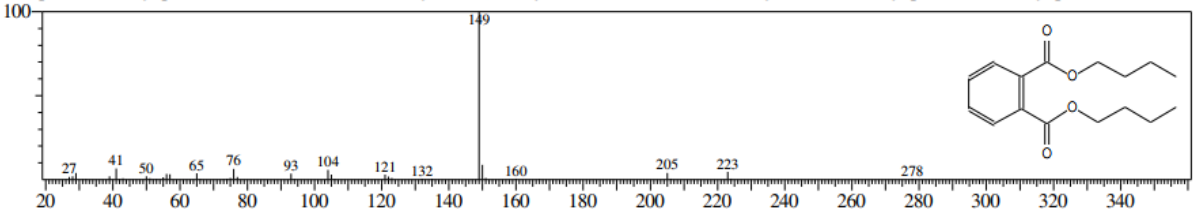
Hit#:2 Entry:173393 Library:NIST11.lib
SI:86 Formula:C27H56 CAS:0-00-0 MolWeight:380 RetIndex:2641
CompName:2-methylhexacosane



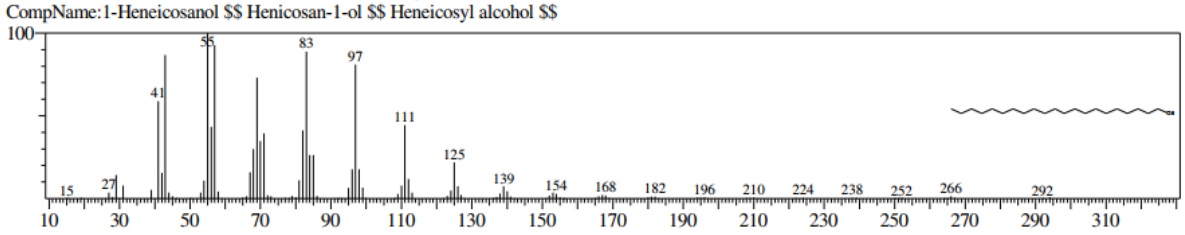
Hit#:14 Entry:252628 Library:WILEY8.LIB
SI:91 Formula:C22H46 CAS:629-97-0 MolWeight:310 RetIndex:0
CompName:DOCOSANE $$ C22H46 STANDARD $$ EINECS 211-121-5 $$ N-DOCOSANE $$ NORMAL-DOCOSANE $$ NSC 77139



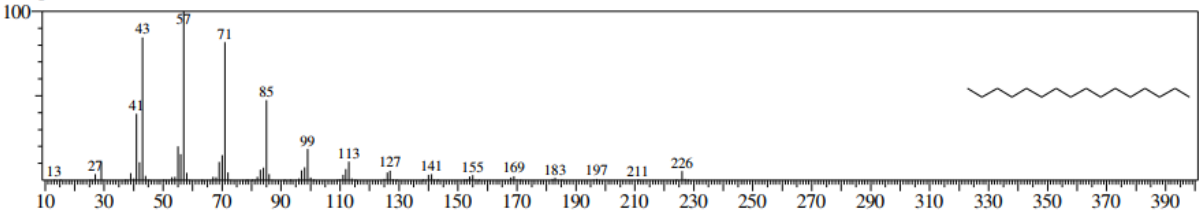
Hit#:1 Entry:101150 Library:NIST11.lib
SI:97 Formula:C16H22O4 CAS:84-74-2 MolWeight:278 RetIndex:2037
CompName:Dibutyl phthalate $$ 1,2-Benzenedicarboxylic acid, dibutyl ester $$ Phthalic acid, dibutyl ester $$ n-Butyl phthalate $$ Butyl phthalate $$ Cellu



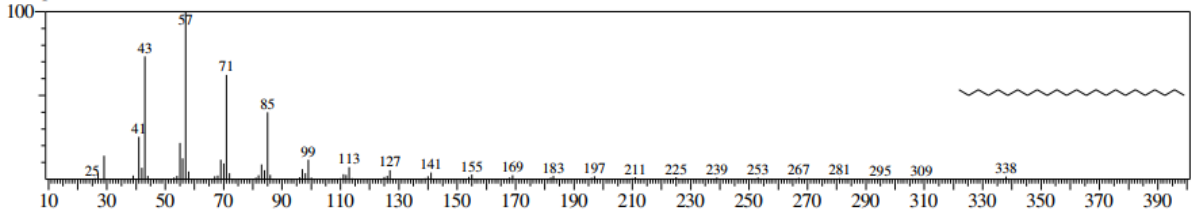
Hit#:7 Entry:128253 Library:NIST11.lib
SI:94 Formula:C21H44O CAS:15594-90-8 MolWeight:312 RetIndex:2351
CompName:1-Heneicosanol $$ Henicosan-1-ol $$ Heneicosyl alcohol $$



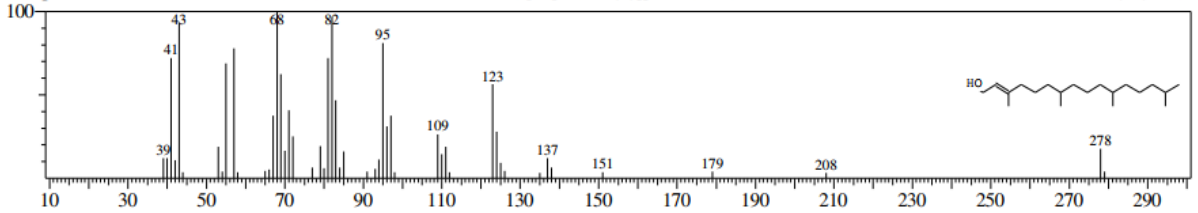
Hit#:7 Entry:20750 Library:NIST11s.lib
SI:92 Formula:C16H34 CAS:544-76-3 MolWeight:226 RetIndex:1612
CompName:Hexadecane $$ n-Cetane $$ n-Hexadecane $$ Cetane $$



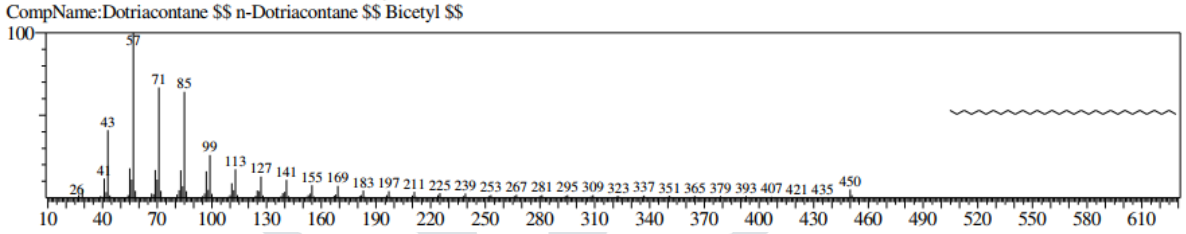
Hit#:16 Entry:28019 Library:NIST11s.lib
SI:91 Formula:C24H50 CAS:646-31-1 MolWeight:338 RetIndex:2407
CompName:Tetracosane $$ n-Tetracosane $$



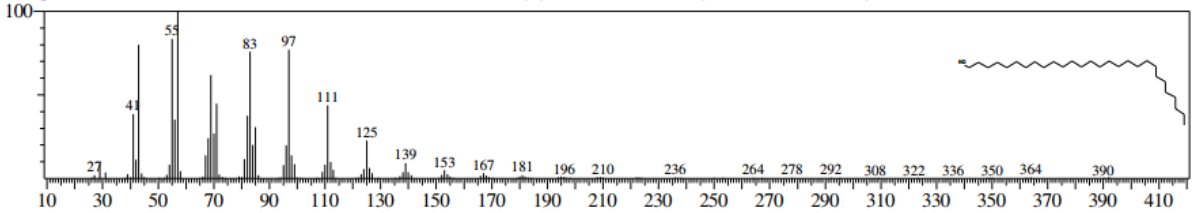
Hit#:3 Entry:235588 Library:WILEY8.LIB
SI:91 Formula:C20H40O CAS:150-86-7 MolWeight:296 RetIndex:0
CompName:2-HEXADECEN-1-OL, 3,7,11,15-TETRAMETHYL-, [R-[R*,R*-(E)]]- $$ 3,7,11,15-TETRAMETHYLHEXADEC-2-EN-1-OL $$ (2E)-3,7,1



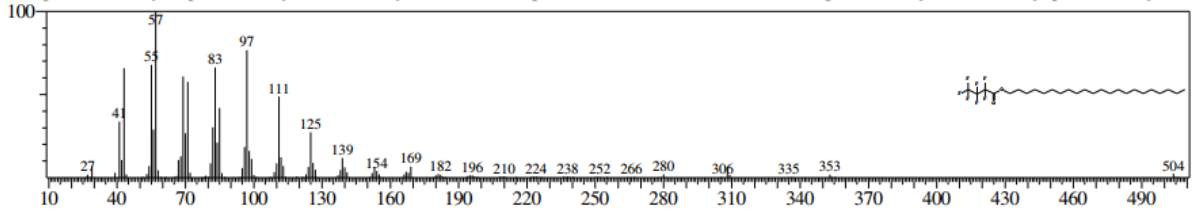
Hit#:8 Entry:30293 Library:NIST11s.lib
SI:88 Formula:C32H66 CAS:544-85-4 MolWeight:450 RetIndex:3202
CompName:Dotriacontane $$ n-Dotriacontane $$ Bicetyl $$

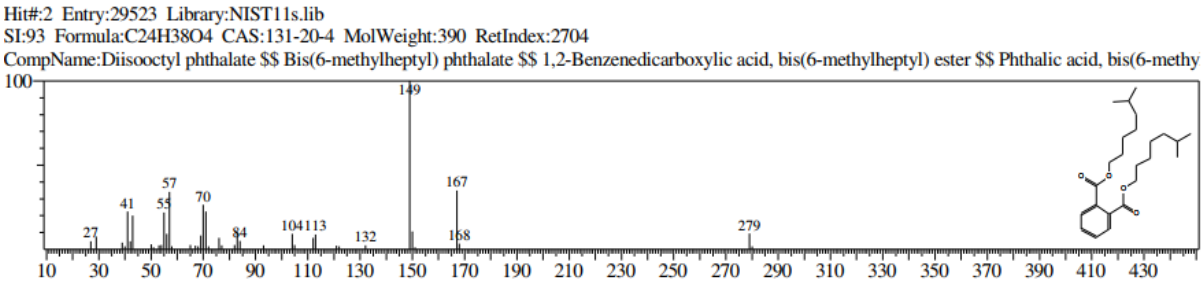


Hit#:3 Entry:29853 Library:NIST11s.lib
SI:92 Formula:C28H58O CAS:557-61-9 MolWeight:410 RetIndex:3047
CompName:Octacosanol $$ 1-Octacosanol $$ n-Octacosanol $$ Cluytyl alcohol $$ Montanyl alcohol $$ Octacosyl alcohol $$



Hit#:19 Entry:206638 Library:NIST11.lib
SI:90 Formula:C26H45F7O2 CAS:0-00-0 MolWeight:522 RetIndex:2330
CompName:Docosyl heptafluorobutyrate $$ Docosyl 2,2,3,3,4,4,4-heptafluorobutanoate $$ 1-Docosanol, heptafluorobutyrate $$ Docosyl perfluorobutyrate





1. The peak at 14.072
The molecular formula of the compound is $C_{20}H_{42}$
The m/z peak at 26, 41, 43, 50, 71, 85, 99,113, 127, 141, 155, 169, 183, 197, 211, 225, 239, 253 and 282 favored the presence of **Eicosane**.

2. The peak at 14.389
The molecular formula of the compound is **$C_{14}H_{22}O$**
The m/z peak at 15,27,41,55,57,77,91,107,115,135,147,163,175,191 and 206 favored the presence of **Phenol, 3,5-bis(1,1-dimethylethyl**).

3. The peak at 14.655
The molecular formula of the compound is **$C_{18}H_{38}$**
The m/z peak at 21, 41, 43, 57, 71, 85, 99,113, 127, 141, 155, 169, 183, 197, 211 and 254 favored the presence of **Octadecane.**

4. The peak at 15.307
The molecular formula of the compound is **$C_{16}H_{34}O$**
The m/z peak at 39, 41, 55, 69, 83, 97,111, 125, 140, 154, 168, 196 and
224 favored the presence of **1-Hexadecanol.**

5. The peak at 16.592
The molecular formula of the compound is **$C_{15}H_{32}$**
The m/z peak at 15, 27, 41, 43, 57, 71, 85, 99,113, 127, 141, 155, 169, 183, 197, and 212 favored the presence of **Pentadecane.**

6. The peak at 16.756
The molecular formula of the compound is **$C_{16}H_{34}$**
The m/z peak at 13, 27, 41, 43, 57, 71, 85, 99,113, 127, 141, 155, 169, 183, 197, 211, and 226 favored the presence of **Hexadecane.**

7. The peak at 17.108
The molecular formula of the compound is **$C_{11}H_{24}O$**
The m/z peak at 15, 27, 41, 43, 57, 71, 85, 111, 125, 126, 141 and 172 favored the presence of **2-Isopropyl-5-methyl-1-heptanol.**

8. The peak at 17.256
The molecular formula of the compound is **$C_{21}H_{44}$**
The m/z peak at 24, 41, 43, 57, 71, 85, 99,113, 127, 141, 155, 168, 182, 196, 210, 224, 240, 265 and 283 favored the presence of **Pentadecane, 8-hexyl.**

9. The peak at 17.686
The molecular formula of the compound is **$C_{17}H_{36}O_{23}$**
The m/z peak at 15, 27, 41, 56, 57, 83, 97,111, 125, 126, 140, 154, 168, 182, 196, 210 and 238 favored the presence of **n-Heptadecanol-1**.

10. The peak at 18.223
The molecular formula of the compound is **$C_{20}H_{38}$**
The m/z peak at 27, 41, 43, 68, 82, 95, 109, 123, 137, 151, 179, 193, 208, 263 and 278 favored the presence of **2, 6, 10-Trimethyl, 14-Pentadecne.**

11. The peak at 18.319
The molecular formula of the compound is **$C_{25}H_{52}$**
The m/z peak at 27, 41, 43, 57, 71, 85, 99,113, 127, 141, 155, 169, 183, 197, 211, 225, 239, 253, 267, 309 and 337 favored the presence of **2-Methyltetracosane.**

12. The peak at 18.710
The molecular formula of the compound is **$C_{16}H_{22}O_4$**
The m/z peak at 41, 43, 57, 76, 93,104, 121, 132, 149, 167, 205 and 223 favored the presence of **1, 2-Benzene Dicarboxylic Acid**.

13. The peak at 18.850
The molecular formula of the compound is **$C_{21}H_{44}$**
The m/z peak at 15, 26, 41, 43, 57, 71, 85, 99,113, 127, 141, 155,
169, 183, 197, 211, 225, 239, 253, 267, 281 and 296 favored the presence of **Heneicosane.**

14. The peak at 19.157
The molecular formula of the compound is **$C_{34}H_{70}$**
The m/z peak at 18, 41, 43, 57, 71, 85, 99,113, 127, 141, 155, 169, 183, 197 and 211 favored the presence of **Tetratricontane**

15. The peak at 19.353
The molecular formula of the compound is **$C_{17}H_{24}O_3$**
The m/z peak at 51, 55, 57, 77, 91,109, 119, 135, 149, 161, 175, 189, 205, 217, 232, 261 and 276 favored the presence of **7,9-Di-tetra-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione.**

16. The peak at 19.517
The molecular formula of the compound is **$C_{27}H_{56}$**
The m/z peak at 27, 41, 43, 57, 71, 85, 99,113, 127, 141, 155, 169, 183, 197, 211, 225, 239, 253, 267, 281, 295, 309, 337, and 365 favored the presence of **2-methylhexacosane**
17. The peak at 19.593
The molecular formula of the compound is **$C_{22}H_{46}$**
The m/z peak at 39, 41, 43, 57, 71, 85, 99,113, 127, 141, 155, 169, 183, 197, 211, 225, 239, 253 and 310 favored the presence of **Docosane**.

18. The peak at 19.726
The molecular formula of the compound is **$C_{16}H_{22}O_4$**
The m/z peak at 27, 41, 50, 65, 76, 93,104, 121, 132, 149, 160, 205, 223 and 278 favored the presence of **Dibutyl phthalate**.

19. The peak at 19.837
The molecular formula of the compound is **$C_{21}H_{44}O$**
The m/z peak at 15, 27, 41, 56, 83, 97,111, 125, 139, 154, 168, 182, 196, 210, 224, 238, 252, 266 and 292 favored the presence of **1-Heneicosanol**.

20. The peak at 19.892
The molecular formula of the compound is **$C_{16}H_{34}$**
The m/z peak at 13, 27, 41, 43, 57, 71, 85, 99,113, 127, 141, 155, 169, 183, 197, 211and 226 favored the presence of **Hexadecane**

21. The peak at 20.900
The molecular formula of the compound is **$C_{24}H_{50}$**
The m/z peak at 25, 41, 43, 57, 71, 85, 99,113, 127, 141, 155, 169, 183, 197, 211, 225, 281, 295, 309 and 338 favored the presence of **Tetracosane.**

22.The peak at 21.169
The molecular formula of the compound is **$C_{20}H_{40}O$**
The m/z peak at 39, 41, 43, 68, 82, 95, 109, 123, 137, 151, 179, 208 and 278 favored the presence of **2-hexadecen-1-OL, 3, 7, 11, 15-Tetramethyl**.

23. The peak at 21.376
The molecular formula of the compound is **$C_{32}H_{66}$**
The m/z peak at 26, 41, 43, 50, 71, 85, 99,113, 127, 141, 155, 169, 183, 197, 211, 225, 239, 253, 267, 281, 295, 309, 323, 337, 351, 365, 379, 393, 407, 421, 435 and 450 favored the presence of **Dotriacontane.**

24. The peak at 21.975
The molecular formula of the compound is **$C_{28}H_{58}O$**
The m/z peak at 27, 41, 55, 83, 97,111, 125, 139, 153, 167, 181, 196, 210, 236, 264, 278, 292, 308, 322, 336, 350, 364 and 390 favored the presence of **Octacosanol.**

25. The peak at 21.927
The molecular formula of the compound is **$C_{26}H_{45}F_7O_2$**
7, 41, 55, 57, 83, 97,111, 125, 139, 154, 169, 182, 196, 210, 224, 238, 252, 266 and 280 favored the presence of **Docosyl heptafluorobutyrate.**

26. The peak at 28.675
The molecular formula of the compound is **$C_{24}H_{38}O_4$**
The m/z peak at 27, 41, 55, 57, 70, 84, 104, 133, 132, 149, 167, 168 and 279 favored the presence of **Diisooctyl phthalate.**

IV CONCLUSION

The present study was undertaken to purify and characterize the active constituents of *Phyla nodiflora* L. extracted with organic solvents using TLC and Column chromatography.
The chloroform extract revealed 5 bands and further purified using HPLC-MS and further subjected to GC-MS analysis. The GC-MS analysis favored the presence of 26 compounds at different RT which are reported for the first time in *Phyla nodiflora*. The predominant peak at 14.389 indicate the presence of Phenol, 3,5-bis (1,1-dimethylethyl), peak at 18.710 indicate the presence of 1,2- Benzenedicarboxylic acid, bis, peak at 19.667 indicate the presence of Dibutyl phthalate.

V ACKNOWLEDGEMENT

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REFERENCES

1. Maher G, Enas A, Hane A, Maisa A, Jafar E, Isamel O: Antimicrobial actitivity of cured extracts of some plant leaves. Research Journal of Microbiology 2012; 7: 1-10.
2. Kumar RA, Sridevi K, Kumar VN, Nanduri S, Rajagopal S: Anticancer and immune stimulatory compounds from *Andrographis paniculata*. Journal of Ethnopharmacology 2004; 92: 2-3.
3. Dhanaukar SA, Kulkarrni RA, Rege NN: Pharmaccology of medicinal plants and natural products. Indian journal of pharmacology 2000; 32.81-118.
4. Terblanche FC, Kornelius G*:* Essential oil constituents of the genus *Lippia* (Verbenaceae) a literature review. J. Ess Oil 1996; 8: 471-485.
5. Sharma RA, Renu S: A Review on *Phyla nodiflora* Linn.: A Wild Wetland Medicinal Herb Int. J. Pharm. Sci. Rev. Res 2013; 20: 57-63
6. Pooley E: A Field Guide to Wild Flowers Kwazulu-Natal and Eastern Region. Flora Publications Trust. 1998; 2:1-3.
7. Agarwal RR, Dutt SS: Chemical examination of punarnava or *Boerhaavia diffusa* Linn. Isolation of an alkaloid punarnavine. Chemical Abstract 2001; 30: 3585.
8. Sudha Srinivasan L: Physicochemical and phytochemical profiles of aerial parts of *Lippia Nodiflora* L. IJPSR, 2013; 4: 4263-4271.
9. Harbone J, Parr GR, Smith LM: Gas chromatography analysis-Anal. Chem 1975; 65:3204-3211.
10. Reber EA: Gas Chromatography-Mass Spectrometry (GC-MS): Applications in Archaeology. Encyclopedia of Global Archaeology. Springer, Cham 2018; 304-342.
11. David S: Consultant, and Doug Agnew NIST/EPA/NIH Mass Spectral Library, NIST 98 O Agilent Technologies Co., Palo Alto, CA 1999; 22. 5967-679.
12. Nutan K, Chaturvedi A, Upadhyay RK: Corrosion Inhibition of Mild Steel by Alkaloid Extract of *Ocimum Sanctum* in HCl and HNO3 Solution, Research Journal of Chemical Sciences 2012; 2: 51-56.
13. Balamurupan R, Duraipandiya V, Savarimuthu IG: Antidiabetic activity of Y-Sitosterol isolated from *Lippia Nodiflora L.* in streptozotocin induced diabetic rats." European Journal of pharmacology 2011; 667: 410-418.
14. Wang P: A statistical method for chromatographic alignment of LC- MS DATA. *Biostatistics* 2007; 8: 357-367.