# Chemical and Mass Spectra Analysis of Fractions obtained from Methanol Leaf Extract of *Corymbia torelliana* with *Trypanosoma congolense* effect

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**Abstract:** The use of drugs for the prevention and treatment of trypanosomiasis has been important for many decades, although trypanosomes have developed resistance to each drug introduced. This study is aimed at exploring Chromatography techniques to proffer solution for antitrypanosomal action from the leaves of *Corymbia torelliana*. The Chemical and Mass Spectra Analysis of compounds identified in the methanol leaves extract of *Corymbia torelliana* with *Trypanosoma congolense* effect was achieved using Column Chromatography, Thin Layer Chromatography and Gas Chromatography-Mass Spectral analysis. Column Chromatography was ran to obtain the separated fractions, which was pooled together for Thin Layer Chromatography. The GCMS analysis help to identify the compound by showing their molecular masses, fragmentation patterns, thermostatically molecular mass ions and the major ions of the fragments while the TLC was employed to locate the positions of various fractions as it untie the compounds identification in the system. These compounds are: 2(4H)- Benzofuranone, 5, 6, 7, 7a-tetrahydro-4, 4,7a-trimethyl; 2-Naphthalene methanol, decahydro-alpha, alpha, 4a-trimethyl-8-methylene; Octanal, 2-(phenyl methylene); Hexadecanoic acid, methyl ester; Hexadecanoic acid; 1, 2, 3-Benzenetriol; 3, 5-Di-tert-butylphenol; Benzoic acid, 2-hydroxy-phenylmethyl ester; Hexadecanoic acid; Octadecanoic acid; 9,12,-Octadecadienoic acid methyl ester; 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl; Tetradecanoic acid, methyl ester; 11-Octadecenoic acid, methyl ester; 5-(p-tert-Butylphenoxymethyl)-3-(2-pyridyl)-2-oxazolidone and 1,2-Benzenedicarboxylic acid, dioctyl ester. The presence of these compounds suggest potentials of the leaves of *Corymbia torelliana* plant for possible uses as antitrypanosomal agent, anti-oxidant, anti-cancer, anti-tumour, anti-inflammatory, insectifuge and remedy for mood and sleep disorder.

[Ogbole, E., Daben J.M., Dashak, D.A. Nvau, J.B., Ekwenchi, M.M., Ikyase C., and Igweh, A.C. **Chemical and Mass Spectra Analysis of Fractions obtained from Methanol Leaf Extract of *Corymbia torelliana* with *Trypanosoma congolense* effect.** *N Y Sci J* 2017;10(5):60-75]. ISSN 1554-0200 (print); ISSN 2375-723X (online). http://www.sciencepub.net/newyork. 10. doi:10.7537/marsnys100517.10.

**Key words:** *Corymbia torelliana*, Chromatography techniques, Methanol leaves extract, Mass spectra, GC-MS analysis, Anti-trypanosomal action.

## 1. Introduction

Trypanosomiasis which is caused by different species of Trypanosomes is a severe parasitic disease and has devastifying effects on both human and livestock (Chammond *et al.,* 2010; Adams *et al.,* 2010). Human African Trypanosomiasis (HAT) is commonly called sleeping sickness caused mostly by *Trypanosoma brucei gambiense* which occurs in 36 states of sub-saharan African countries (Simarro *et al.,* 2011; WHO, 2015). Trypanosomiasis and other neglected tropical diseases constitute great disease burden especially in developing countries of the world (Houmsou *et al.,* 2012; Kamga *et al.,* 2012; Daben *et al.,* 2015; Beyene *et al.,*2015).

The disease affects mostly poor population living in the remote rural areas. Regions where the insects are common travelers are at risk. The disease constantly threatens to reach epidemic proportions, as was the case at the beginning of the 20th century (WHO, 2015). The estimation of the numbers and location of undetected and unreported cases, including areas where epidemiological knowledge is limited due to lack of surveillance and accessibility is difficult because of topographic or security constraints. African Animal Trypanosomiasis (AAT) remains one of the major constraints to health and productivity of cattle and other domestic animals in tsetse fly infested areas of tropical Africa (Houmsou *et al.,* 2012). AAT is a major obstacle to economic development of affected rural areas, a serious barrier to human welfare because of the nutritional and economic problems it causes (Daben *et al.,* 2015; WHO, 2015). The use of drugs for the prevention and treatment of trypanosomiasis has been important for many decades, although

trypanosomes have developed resistance to each drug introduced (Essam *et al.,* 2009; Kamga *et al.,* 2012; Beyene *et al.,* 2015). Pathogenic trypanosomes like *T. congolense* infections in cattle have been reported to increase trypanocidal drug use and consequently accelerated the development of resistance against trypanocidal drugs ((Essam *et al.,* 2009).

*Corymbia* is a remarkable species of the family Myrtaceae, with a large genus of aromatic trees long known to be indigenous to Australia (Adeniyi and Ayepola, 2008), Tasmania and the neighbouring islands but today can be found growing in tropical and subtropical regions of the world (Coffi *et al.,* 2012). This genus, which includes over 800 species ( Pinto *et al.,* 2016) is the second most widely planted multipurpose woody tree species in the world, they occur under a wide range of environmental conditions. The remarkable adaptability of *Corymbia* coupled with their fast growth and superior wood properties has driven their rapid adoption for plantation forestry in more than 100 countries (Ololade and Olawore, 2013).

The essential oils of the *Corymbia* plant parts are most used as a remedy for cold and cough and in pharmaceuticals such as cough syrups (Coffi *et al.,* 2012), lozenges, nasal drops and mouthwash (Coffi *et al.,* 2012; Alian *et al.,* 2012). They also have long history of safe use in food preservation, physiotherapies, pesticides (Chalchat *et al.,* 2000), just as the seeds and the leaves oils have antibacterial activities against enteric pathogens (Farah *et al.,* 2002), antiviral, anti-inflammatory, anti-oxidant, antifungal, antimicrobial, antibiotic and anti-carcinogenic properties (Chalchat *et al.,* 2000; Adeniyi *et al.,* 2006; Ben-Hadj *et al.,* 2011; Gaballero-Gallardo *et al.,* 2011).

*Corymbia torelliana* is one of the notable specie in the genus *Corymbia (Eucalyptus)*, and have drawn researches from Nigeria, Mali, Congo-Brazzavilla and Australia to report the significance of C. *torelliana* essential oils rich natural compounds- hydrocarbon monoterpenol, spatulenol, $\alpha$ and $\beta$-pinenes, ocimene, aromadendrene and caryophyllene oxide as its characteristic constituents (Elaissi *et al.,12:81* ). Dashak *et al.,* (2016) reported some compounds that suggest the potentials of the leaves, seed buds and fruits of *Corymbia torelliana* plant for possible uses as antiseptics, disinfectants food additives. Ogbole *et al.,* (2017) had reported the leaves for antitrypanosomal action.

Other uses in Nigeria include gastrointestinal disorders; the decoction of the leaves has been reported for sore throat remedy and other bacterial infections of respiratory and urinary tract (Adeniyi *et al.,* 2006). The poultice of the leaves is applied over wounds and ulcers (Alian *et al.,* 2012) the leaves extract also decreases gastric acid production and used for the treatment of gastric and duodenal ulcers, cough associated with most pulmonary diseases (Farah *et al.,* 2002; Adeniyi *et al.,* 2006; Alian *et al.,* 2012).

In our search for complementary and alternative medicine through chromatographic techniques as the plant have been documented with preference for the management of anti-tumour, anti-cancer, antiviral, antibacterial, antibiotic, antioxidant and antifungal. Some of these activities have been screened for trypanosomal action. Chromatographic technique in it many forms is widely used as separative and analytical technique that has helped to expatiate the natural components ready to diagnose trypanosomes. To proffer solutions, chromatographic techniques have been used to advance this search.

This work is aimed at identifying the effect of the compounds on *Trypanosoma congolense,* giving the mass spectra of compounds obtained from the Gas Chromatography-Mass Spectrometry analysis and showing possible mechanisms of the mass of fragment lost from the fragmentation patterns of the methanol leaves extract of *C. torelliana.*

## 2.0 Materials and Methods

### 2.1. Collection and Identification of Plant Samples

Fresh leaves of the plant, *Corymbia torelliana* (blood-leaf gum) were obtained in the paddock of the Nigerian Institute for Trypanosomiasis Research (NITR), Vom, Jos, Plateau State, in the month of March 2014. It was identified, Voucher No. FHJ 028, authenticated and deposited in the Herbarium, Department of Horticulture, Federal College of Forestry, Jos, Nigeria. The samples were stored in plastic containers and brought to National Veterinary Research Institute (NVRI), Vom laboratory for analysis.

### 2.2. Preparation of Plant Material

The plant leaves were cleaned and air-dried to constant weight under shade, pulverized into a fine powder with mortar and pestle and then stored in a plastic container for use when required.

### 2.3. Extraction and Concentration of the Samples

Three hundred grams (300g) of the powdered samples was dissolved in 1 litre of 98 % methanol. The mixture was dissolved to stand for 48 hours. The marc was separated from the solvent by decanting and filtration using a clean piece of muslin cloth and subsequently with Whatman filters paper No. 1. Additional fresh solvent was added to the marc, it was occasionally agitated and the extraction process continued daily for five days until for the maximum extraction of the chemical constituents. The filtrate was pooled together and concentrated using vacuum rotary evaporator (R-205) at $35^0$C.

**2.4. Column Chromatography Analysis**

Twenty grams (20g) of the crude extract was dissolved in 10ml of methanol and ethyl acetate which was then adsorbed on 45g of silica gel. The slurry was prepared using 180g of the adsorbent (Silica gel 70-200 mesh) in 500 ml n-hexane and it was gently poured into the column, ensuring no air bubbles were trapped. The packed column was allowed to settle evenly. And the sample was applied on top of the column allowing running through the column.

A steady eluate flow was maintain ed through the column during the complete period of the separation by gravitational feed. The methanol extract was separated using n-hexane: ethyl acetate mobile phase in the ratios (100:0, 95:5, 80:20, 60:40, 40:60, and 20:80) obtaining fractions (F1-F7, F8-F14, F15-F21, F22-F28, F29-F35 and F36-F44) respectively. Similarly Ethyl acetate: methanol mobile phase was also used in the ratio (100:0, 90:10, 70:30, 50:50) obtaining fractions (F45-50, F51-56, F57-F62, F63-68) respectively and methanol mobile phase 100:0 obtaining fractions F69-F75. The effluent was collected in small fractions of 100 ml each in 250 ml beakers.

**2.5. Thin Layer Chromatography Analysis**

The activated silica gel pre-coated plates were spotted with the separated fractions using micro pipette and was allowed to dry. The plates were transferred into the developing tank already saturated with the mobile phases of varying polarity ratio (n-hexane: ethyl acetate; 9:1, 4:1, 3:1, 1:1) and (Chloroform: Methanol; 9:1) for the fractions. The mobile phase for each run was allowed to rise to about 18cm from the origin of the plates, then removed from the tank, allowed to dry and the separated components were viewed under uv-light and their positions circled for determination of their $R_f$ values (Harborne, 1991). The fractions were pooled together according to their TLC profiles. Fractions F4, F10 and F11 were found to show anti-*trypanosoma congolense* activity (Ogbole *et al.,* 2017) and were analyzed using IR and GC-MS spectral analysis.

**2.6. Chemical Analysis of Extracts**

Gas Chromatography and Mass-Spectrophotometer Analysis.

Analysis of the leaves of *Corymbia torelliana* using Gas Chromatography and Mass-Spectrophotometer (Shimadzuma Japan QP2010 PLUS); under the following conditions: AOC-20i auto-injection, column flow rate 1.58ML/ min, injection volume of 1 $\mu$ L at $250^0$C with initial temperature of column at $80^0$C, pressure of 108pKa, total flow of 6.2mL/min and total run time-28mins. Carrier gas Helium at a constant flow rate of 0.99ml/min.

**Table 1. Thin Layer Chromatographic Profile of Fractions**

| Mobile Phase (v/v) | Ratio | C.C Fractions (F) | T.L.C Pooled Fractions (F') |
|---|---|---|---|
| Hexane – EtOAc | 9: 1 | 1 – 9 | No spot |
| Hexane – EtOAc | 4: 1 | 10 – 14 | F'1 |
| Hexane – EtOAc | 4: 1 | 15 – 16 | F'2 |
| Hexane – EtOAc | 4: 1 | 17− 21 | F'3 |
| Hexane – EtOAc | 3: 1 | 22 – 28 | F'4 |
| Hexane – EtOAc | 1: 1 | 29 – 31 | F'5 |
| Hexane – EtOAc | 1: 1 | 32 – 34 | F'6 |
| Hexane – EtOAc | 1: 1 | 35 – 36 | F'7 |
| Hexane – EtOAc | 1: 1 | 37 – 41 | F'8 |
| Hexane – EtOAc | 1: 1 | 42 – 49 | F'9 |
| Hexane – EtOAc | 1: 1 | 50 −54 | F'10 |
| Hexane – EtOAc | 1: 1 | 55 – 59 | F'11 |
| Hexane – EtOAc | 1: 1 | 60 – 69 | F'12 |
| $CHCl_3$ – MeOH | 9: 1 | 70 – 76 | F'13 |

**2.7. Identification of GC-MS Chromatograms**

Identification of leaves chromatograms were compared with published Electron Impact-Mass Spectral (EI-MS) in the NIST (National Institute of Standards of Technology),Shimadzu's Flavours and Fragrance of Natural Synthetic Compounds (FFNSC), and published spectral data. The retention indices were determined based on a homologous series of n-alkanes internal standard analyzed under the same operating conditions. Calibration based on the Automatic Adjustment of Compound Retention Time (AACRT) function of the GC-MS. Relative concentration of the essential oil component were calculated based on GC peak area with computer matching using NIST libraries provided with computer controlling the GC-MS System. The spectrum of unknown component was compared with the spectrums of known components stored in the libraries. The name,

molecular weight and structure of the components of the test materials ascertained (Silverstein *et al,* 1974; Lee, 1998).

**3.0 Results**

**3.1 Chromatographic Profiles and Percentage Yields of the Fractions.**

The percentage yield of the crude methanol extract was 28.24%. The profile of thin layer chromatography revealed 13 fractions (F'1-F'13) and the percentage yields of the fractions are shown in Tables 1 and 2 respectively.

**Table 2. Percentage Yields of the Fractions**

| Fractions | F'1 | F'2 | F'3 | F'4 | F'5 | F'6 | F'7 | F'8 | F'9 | F'10 | F'11 | F'12 | F'13 | Total yield |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| % Yield | 1.1 | 1.6 | 4.1 | 3.6 | 3.9 | 1.3 | 1.5 | 1.7 | 9.5 | 12.5 | 11.6 | 21.9 | 6.2 | 80.5 |

**3.2 Gas Chromatography-Mass Spectral (GC-MS) Analysis.**

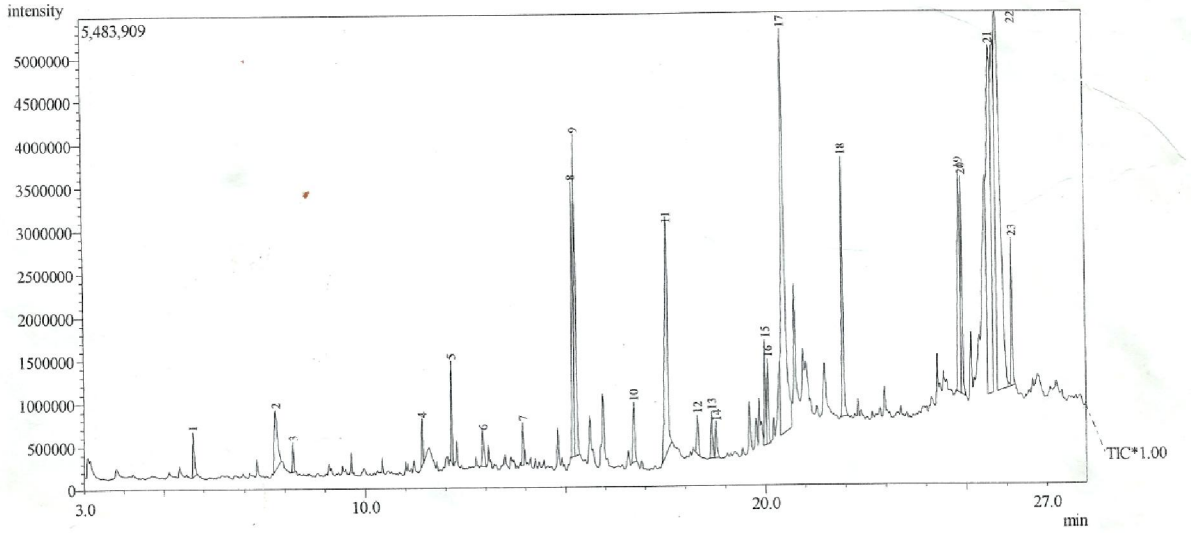



**Figure 1:** GC-MS Chromatograms of F'4. (n- Hexane: Ethyl acetate - 60:40)**3.2.1 Fragmentation Pattern and Structural Elucidation of 2(4H)-Benzofuranone, 5, 6, 7, 7a-tetrahydro-4, 4,7a-trimethyl ($C_{11}H_{16}O_2$)**

R.Time: 11.425mins

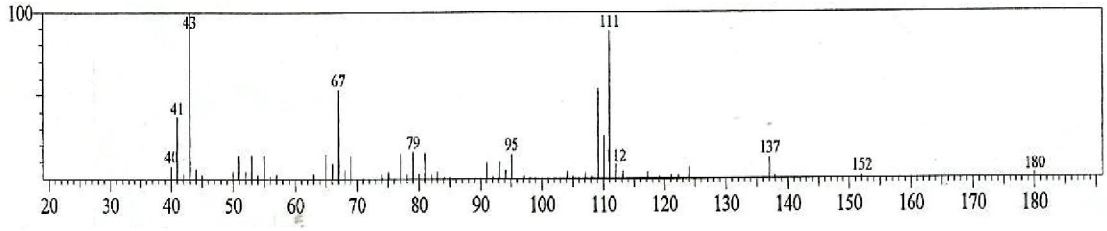
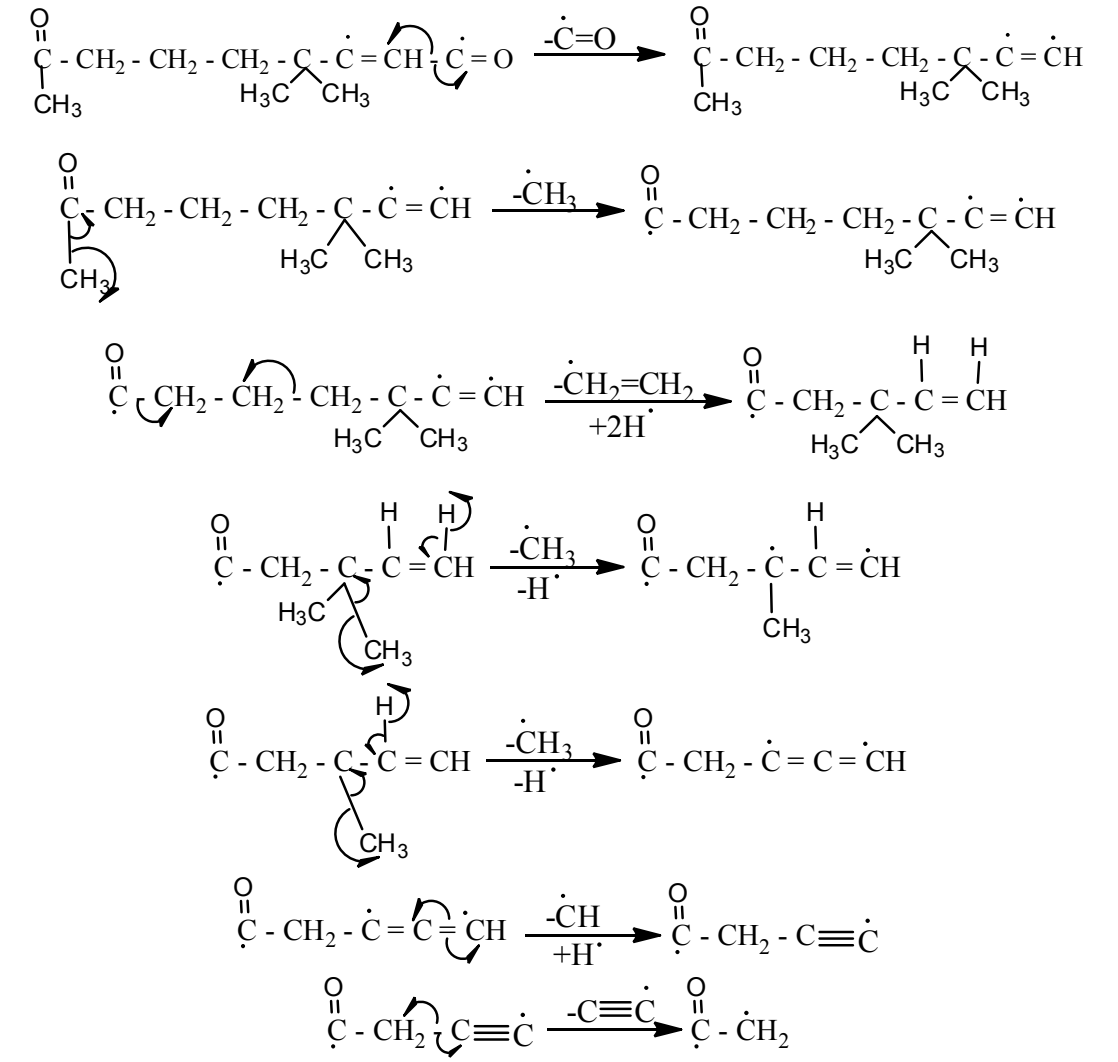
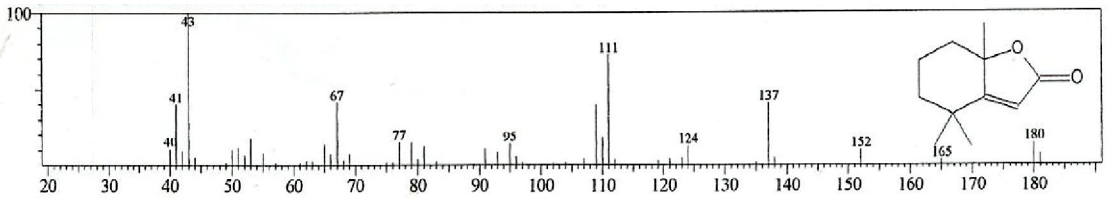



**Figure 2:** Mass Spectrum of Peak 4 of figure 1

The Fragmentation Pattern of 180,152, 137, 111, 95, 79, 67, 43, 41 and 40 in figure 2a has it thermostatically molecular mass ion and major ion of the fragments as 43. The mass of fragments of the molecular ions loss between these fragmentations are: 28(-CO), 15($CH_3$), 26(-$CH_2$=$CH_2$, +2H), 16(-$CH_3$, -H), 16(-$CH_3$, -H), 12(-CH +H), 24(-C≡C), 2(-2H), 1(-1H), as shown possible in the structure of 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl.

Ring opening





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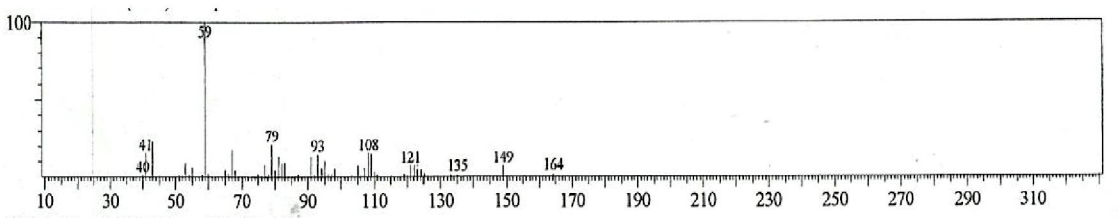
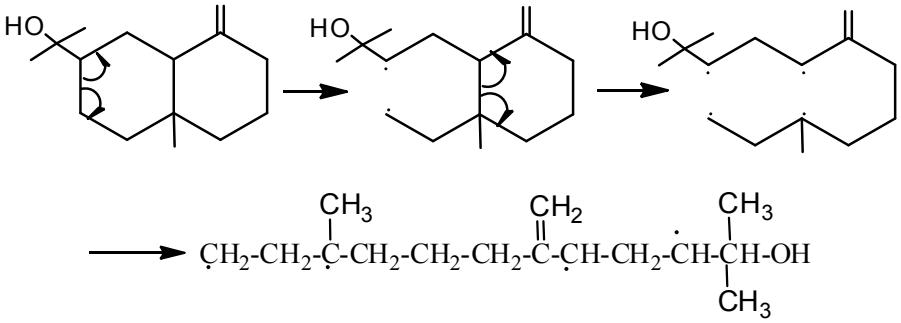


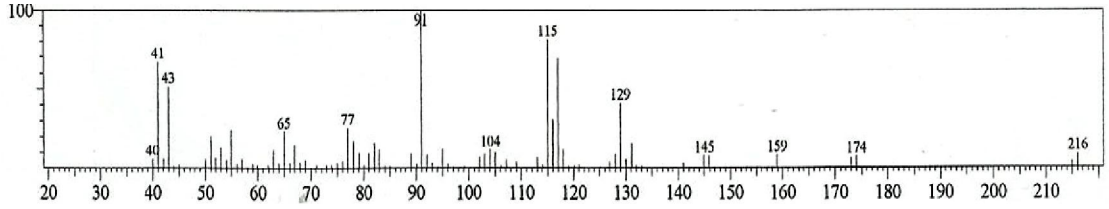
**Figure 3:** Mass Spectrum of Peak 6 of figure 1

The Fragmentation Pattern of 164,149, 135, 121,108, 93, 79, 53, 41 and 40 in figure 3 has it thermostatically molecular mass ion and major ion of the fragments as 53. The mass of fragments of the molecular ions loss between these fragmentations are: 15(-$CH_3$), 14(-$CH_2$), 14(-$CH_2$), 13(-$CH_2$, +H), 15(-$CH_3$), 14(-$CH_2$), 26(-CH≡CH), 12(-$CH_2$, +2H), 1(-H), as shown possible in the structure of 2-Naphthalenemethanol, decahydro-alpha, alpha, 4a-trimethyl-8-methylene.




### 3.2.3 Fragmentation Pattern and Structural Elucidation of Octanal, 2-(phenylmethylene) ($C_{15}H_{20}O$)

R.Time: 13.933mins

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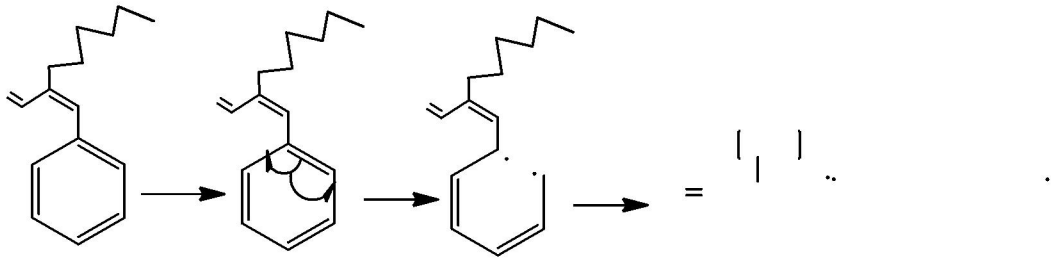


### 3.2.4

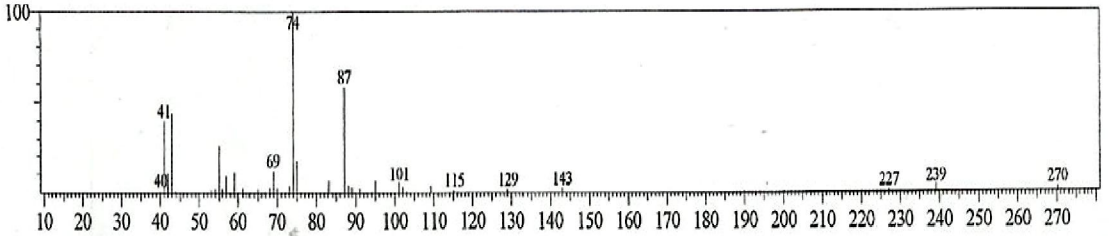
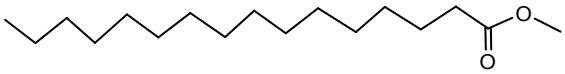



**Figure 5:** Mass Spectrum of Peak 10 of figure 1

The Fragmentation Pattern of 270,239, 227, 143, 129, 115, 101, 87, 74, 69, 41, and 40 in figure 5 has it thermostatically molecular mass ion and major ion of the fragments as 74. The mass of fragments of the molecular ions loss between these fragmentations are: 31(-$OCH_3$), 12(-$CH_2$, +2H), 84(-$(CH_2)_6$, 14(-$CH_2$), 14(-$CH_2$), 14(-$CH_2$), 14(-$CH_2$), 13(-$CH_2$, +H), 5(-5H), 28(-$CH_2$=$CH_2$), 1(-H), as shown possible in the structure of Hexadecanoic acid, methyl ester.



$CH_3$ - $CH_2$ - $CH_2$ - $CH_2$ - $CH_2$ - $CH_2$ - $CH_2$ - $CH_2$ - $CH_2$ - $CH_2$ - $CH_2$ - $CH_2$ - $CH_2$ - $CH_2$ -C(=O)-$OCH_3$

### 3.2.5 Fragmentation Pattern and Structural Elucidation of Hexadecanoic acid ($C_{16}H_{32}O_2$)

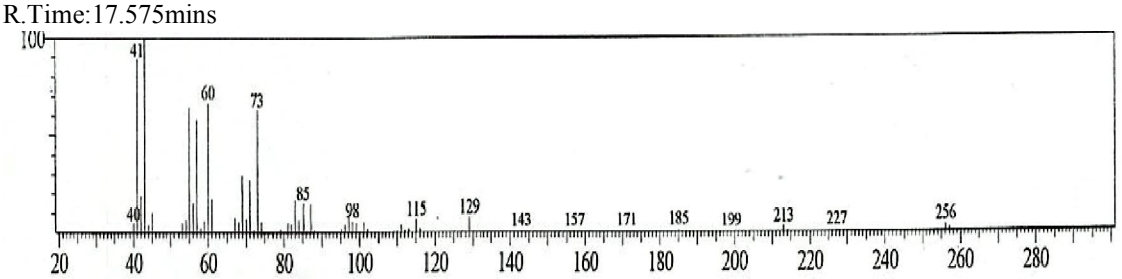
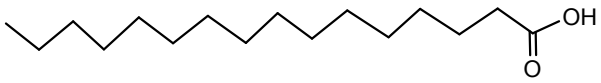



**Figure 6:** Mass Spectrum of Peak 11 of figure 1

The Fragmentation Pattern of 256, 227, 213, 199, 185, 171, 157, 143, 129, 115, 98, 85, 73, 60, 41 and 40 in figure 6 has it thermostatically molecular mass ion and major ion of the fragments as 43. The mass of fragments of the molecular ions loss between these fragmentations are: 29(-$CH_3CH_2$), 14(-$CH_2$), 14(-$CH_2$), 14(-$CH_2$), 14(-$CH_2$), 14(-$CH_2$), 14(-$CH_2$), 14(-$CH_2$), 14(-$CH_2$), 17(-OH), 13(-$CH_2$, +H), 12(-$CH_2$, +2H), 13(-$CH_2$, +H), 19($H_2O$, -H) and 1(-H), as shown possible in the structure of Hexadecanoic acid.



$CH_3$-$CH_2$ $CH_2$-$CH_2$-$CH_2$-$CH_2$-$CH_2$-$CH_2$-$CH_2$-$CH_2$-$CH_2$-$CH_2$-$CH_2$-$CH_2$-C(=O)-OH

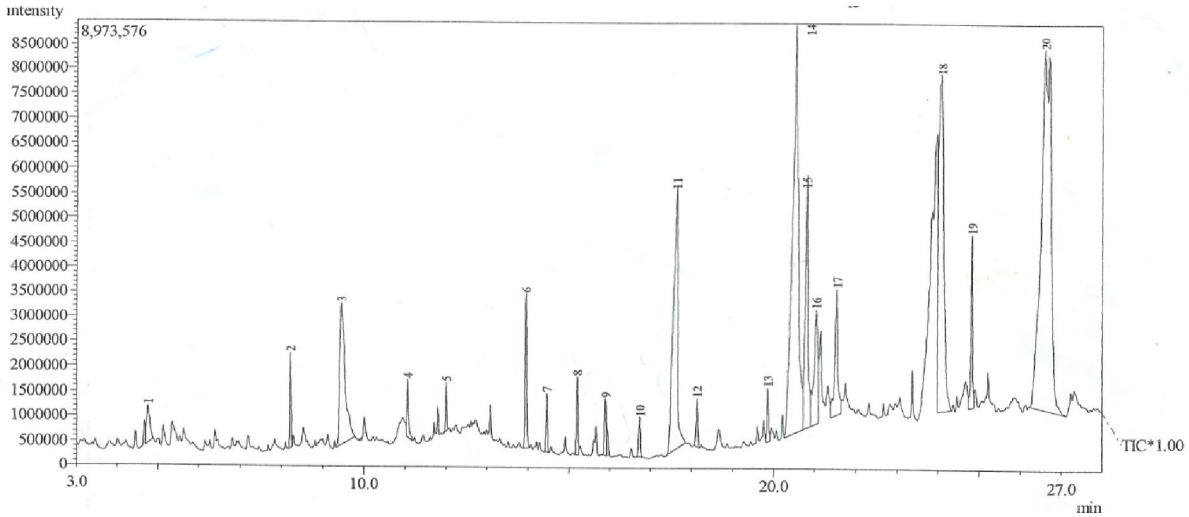
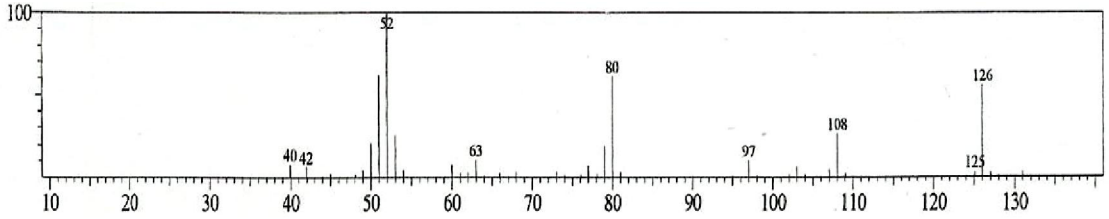



Figure 7: GC-MS Chromatogram of F'10. (Ethyl acetate: Methanol - 70:30)

### 3.2.6 Fragmentation Pattern and Structural Elucidation of 1, 2, 3-Benzenetriol ($C_6H_6O_3$)

R. Time: 9.450mins

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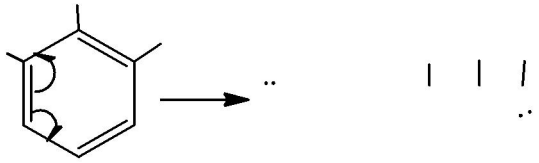

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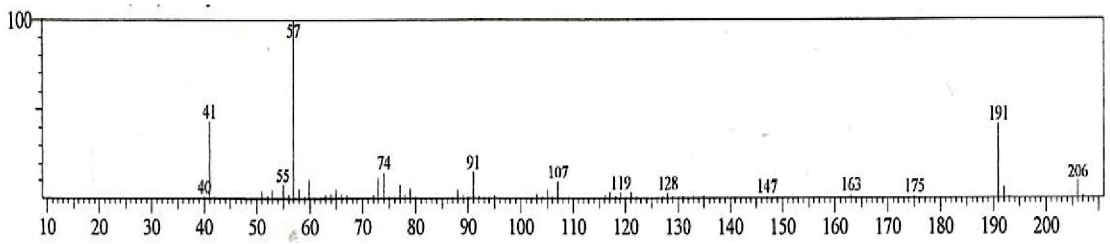
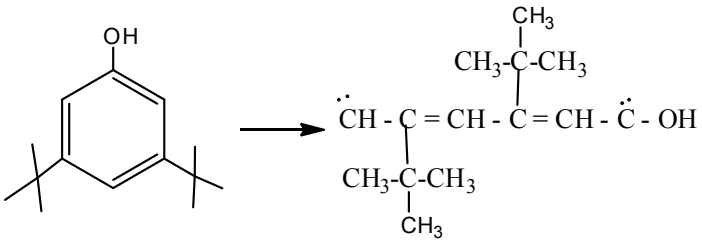


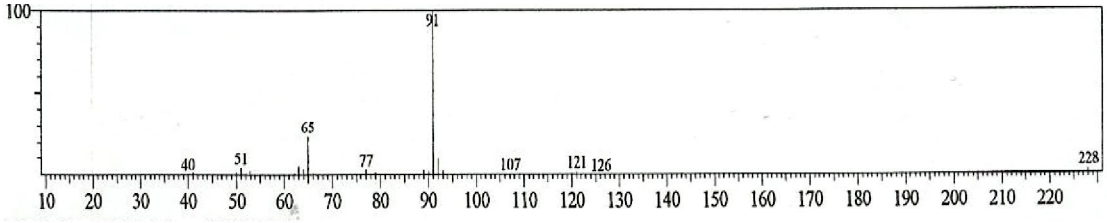

**Figure 9:** Mass Spectrum of Peak 4 of figure

The Fragmentation Pattern of 206, 191, 175, 163,147, 128, 119, 107, 91, 74, 57, 55, 41 and 40 in figure 9 has it thermostatically molecular mass ion and major ion of the fragments as 57. The mass of fragments of the molecular ions loss between these fragmentations are: 15(-$CH_3$), 16(-$CH_3$, -H), 12(-CH, +H), 16(-$CH_3$, -H), 19(-OH, -2H), 9(-9H), 12(-C), 16(-C≡C, +8H), 17(-$CH_3$, -2H), 2(-2H), 14(-$CH_2$), 1(-1H), as shown possible in the structure of 3,5-Di-tert-butylphenol.




### 3.2.8 Fragmentation Pattern and Structural Elucidation of Benzoic acid, 2- hydroxy-phenylmethyl ester ($C_{14}H_{12}O_3$)

R. Time: 15.900mins

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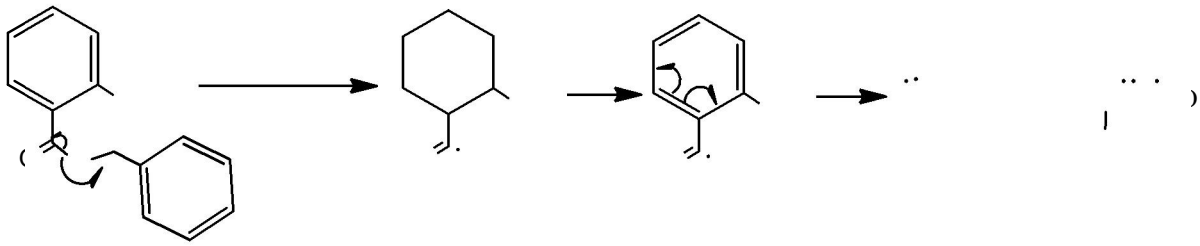

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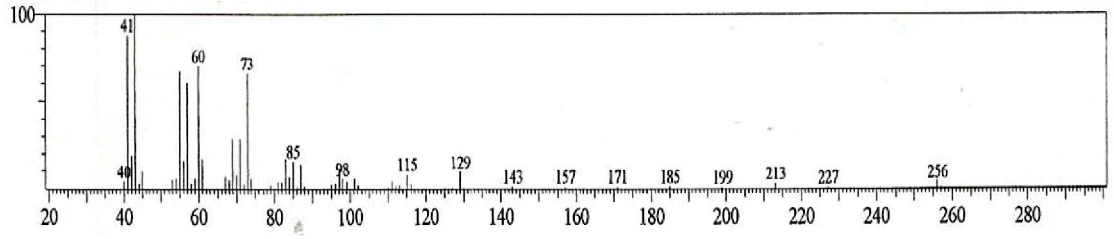


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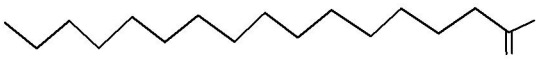

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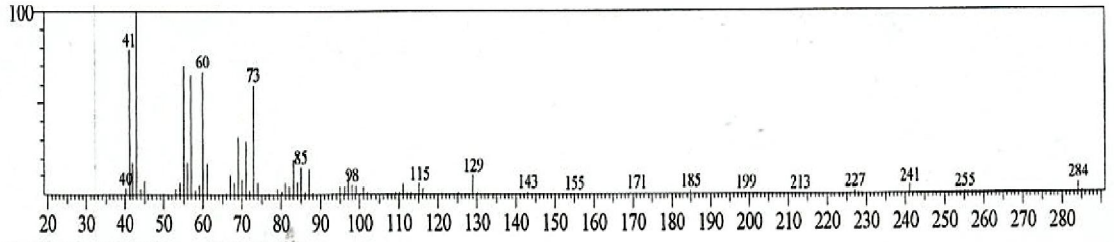
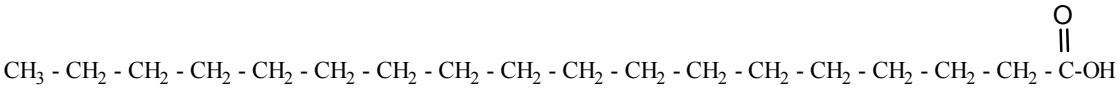



**Figure 12:** Mass Spectrum of Peak 15 of figure 7

The Fragmentation Pattern of 284, 255, 241, 227, 213, 199, 185, 171, 155, 143, 129, 115, 98, 85, 73, 60, 43, 41 and 40 in figure 12 has it thermostatically molecular mass ion and major ion of the fragments as 43. The mass of fragment loss between these fragmentations are: 29(-$CH_3CH_2$), 14(-$CH_2$), 14(-$CH_2$), 14(-$CH_2$), 14(-$CH_2$), 14(-$CH_2$), 14(-$CH_2$), 16(-$CH_3$, -H), 12(-$CH_2$, +2H), 14(-$CH_2$), 14(-$CH_2$), 17(-OH), 13(-$CH_2$, +H), 12(-$CH_2$, +2H), 13(-$CH_2$, +H), 19(-OH, -2H), 1(-H), as shown possible in the structure of Octadecanoic acid.



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### 3.2.11 Fragmentation Pattern and Structural Elucidation of 9, 12,-Octadecadienoic acid, methyl ester

R.Time: 21.042mins

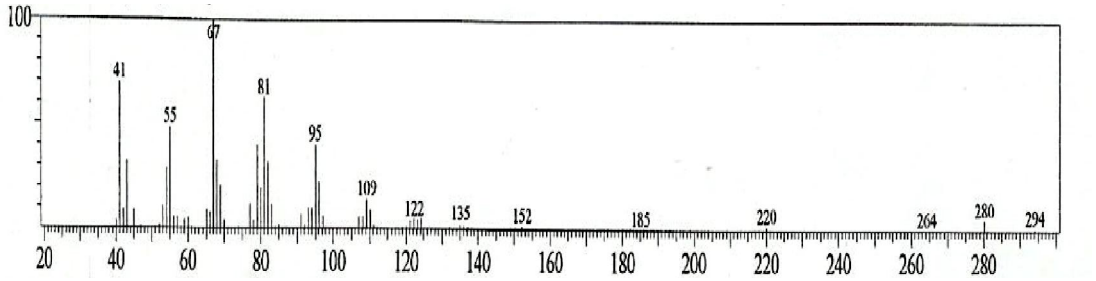
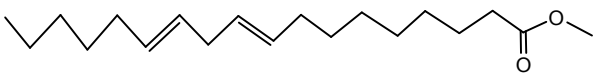



**Figure 13:** Mass Spectrum of Peak 16 of figure 7

The Fragmentation Pattern of 294, 280, 264, 220, 185, 152, 135, 122, 109, 95, 81, 67, 55, 41 in figure 13 has it thermostatically molecular mass ion and major ion of the fragments as 67. The mass of fragments of the molecular ions loss between these fragmentations are: 14(-$CH_2$), 16(-$CH_3$, -H), 44(-$CH_2C$=O, -2H), 35(-$OCH_3$, -4H), 33(-$CH_2$-$CH_2$, -4H), 17(-$CH_3$, -2H), 13(-$CH_2$, +H), 13(-CH), 14(-$CH_2$), 14(-$CH_2$), 14(-$CH_2$), 12(-$CH_2$,+2H), 13(-CH), 14(-$CH_2$), as shown possible in the structure of 9,12,-Octadecadienoic acid, methyl ester.



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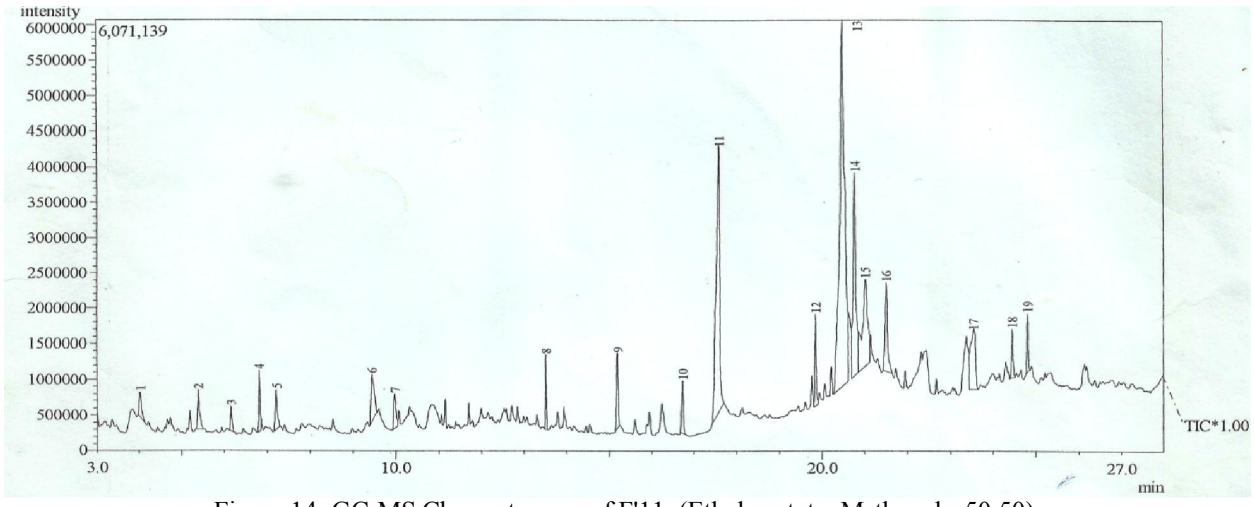
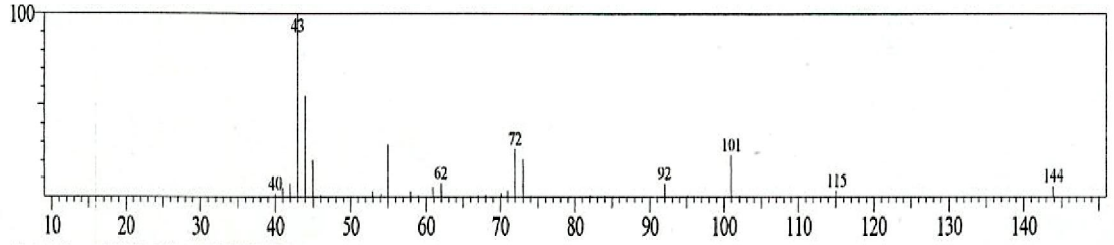



Figure 14: GC-MS Chromatogram of F'11. (Ethyl acetate: Methanol - 50:50)

### 3.1.12 Fragmentation Pattern and Structural Elucidation of 4H-Pyran-4-one, 2,3- dihydro-3,5-dihydroxy-6-methyl ($C_6H_8O_4$)

R. Time: 6.158mins

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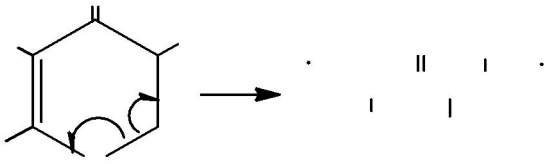

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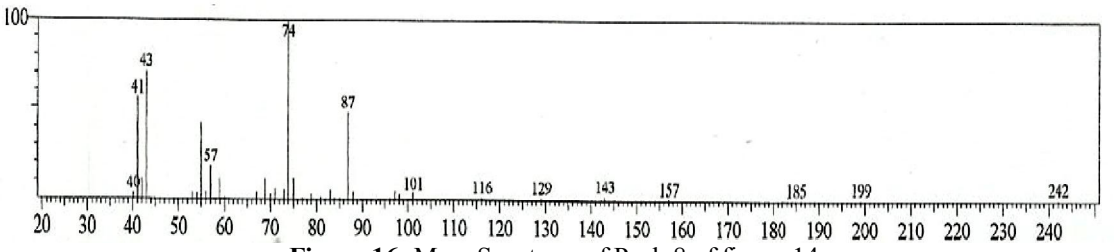
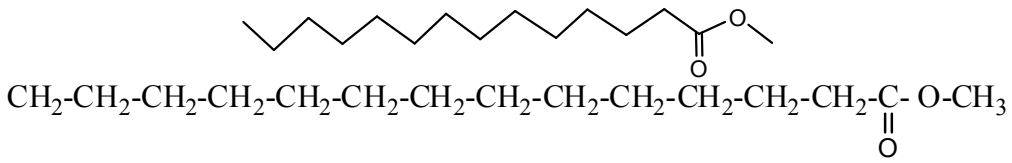


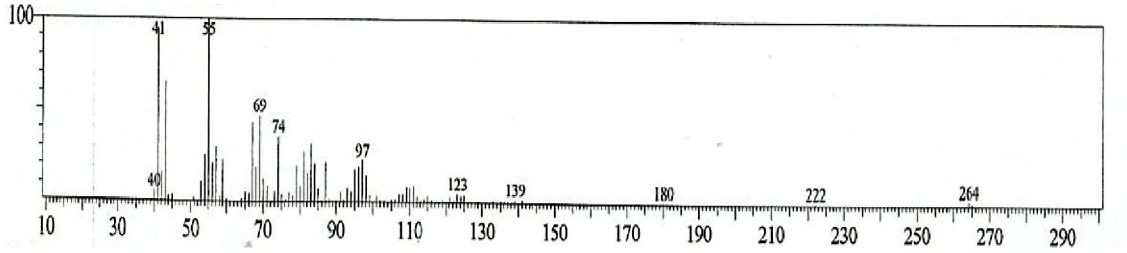

**Figure 16:** Mass Spectrum of Peak 8 of figure 14

The Fragmentation Pattern of 242, 199, 185, 157, 143, 129, 116, 101, 87, 74, 57, 43, 41and 40 in figure 16 has it thermostatically molecular mass ion and major ion of the fragments as 74. The mass of fragments of the molecular ions loss between these fragmentations are: 43(-$C_3H_7$), 14(-$CH_2$), 28(-$CH_2$=$CH_2$), 14(-$CH_2$), 14(-$CH_2$), 13(-$CH_2$,+H), 15(-$CH_3$), 14(-$CH_2$), 13(-$CH_2$, +H), 17(-OH), 14(-$CH_2$), 2(-2H), and 1(-H), as shown possible in the structure of Tetradecanoic acid, methyl ester.




### 3.2.14 Fragmentation Pattern and Structural Elucidation of 11-Octadecenoic acid, methyl ester ($C_{19}H_{36}O_2$)

R. Time: 19.858mins

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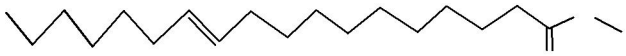

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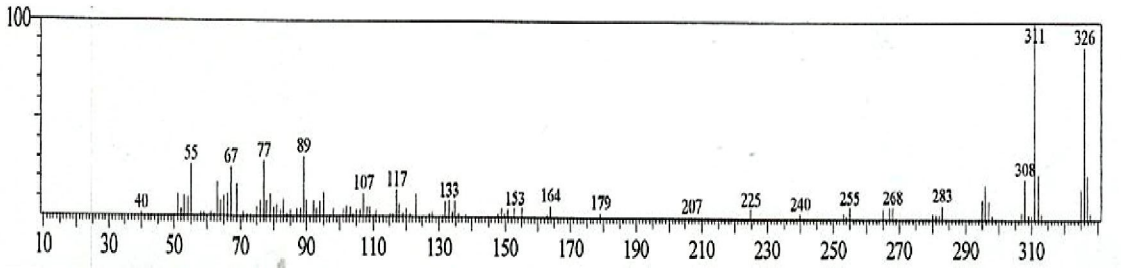
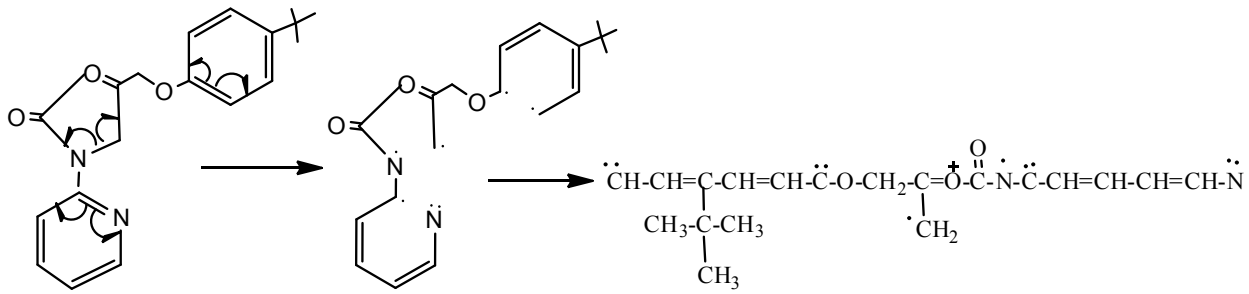



**Figure 18:** Mass Spectrum of Peak 17 of figure 14

The Fragmentation Pattern of 326, 311, 308, 283, 255, 240,225, 207, 164,153, 133, 117, 107, 89, 77, 67, 55 and 40 in figure 18 has it thermostatically molecular mass ion and major ion of the fragments as 311. The mass of fragments of the molecular ions loss between these fragmentations are: 15(-$CH_3$), 3(-3H), 25(-CH≡CH), 28(-$CH_2$=$CH_2$), 15(-$CH_3$), 15(-$CH_2$), 18(-$CH_3$, -3H), 43(-$CH_3$C=O), 11(-$CH_2$), 20(-OH, -2H), 16(-$CH_3$,-H), 28(-$CH_2$=$CH_2$), 12(-$CH_2$, +2H), 10(-$CH_2$, +4H), 12(-$CH_2$,+2H), 15(-$CH_3$), as shown possible in the structure of 5-(p-tert-Butylphenoxymethyl)-3-(2-pyridyl)-2-oxazolidone.



### 3.2.16 Fragmentation Pattern and Structural Elucidation of 1,2-Benzenedicarboxylic acid, dioctyl ester ($C_{24}H_{38}O_4$)

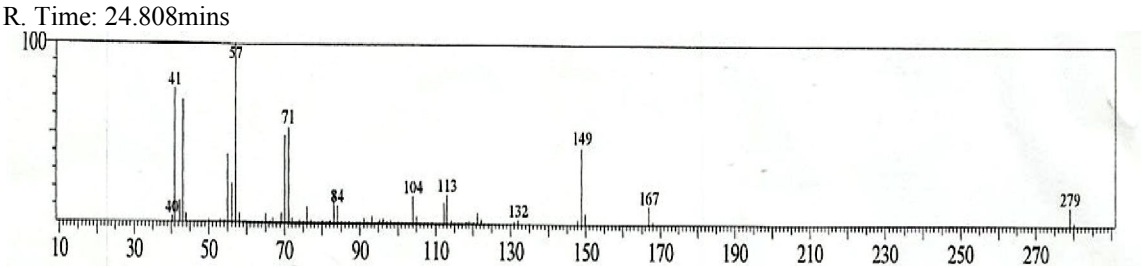



**Figure 19:** Mass Spectrum of Peak 19 of figure 16 The Fragmentation Pattern of 279, 167,149, 132, 113, 104, 84, 71, 57, 41 and 40 in figure 19 has it thermostatically molecular mass ion and major ion of the fragments as 57. The mass of fragments of the molecular ions loss between these fragmentations are: 112(), 18(-$CH_3$, -3H), 17(-OH), 19(-OH, -2H), 9(-9H), 20(-OH, -3H), 13(-$CH_2$, +H), 14(-$CH_2$), 16(-$CH_3$,-H), 1(-H), as shown possible in the structure of 1,2-Benzenedicarboxylic acid, dioctyl ester.

## 4. Discussion

The GC-MS results of Fractions (4, 10 and 11) obtained from Methanol Leaf Extract of *Corymbia torelliana* with anti-*trypanosoma congolense* action revealed the presence of hexadecanoic acid and hexadecanoic acid methyl ester as the common compounds. These compounds have approximately the same retention times, molecular mass, mass of fragment lost, major ion of the fragments, fragmentation pattern and thus the mechanisms. This further confirms that they are the same compound and studies have shown that they are used as anti-oxidant, hypocholesterolemic nematicide, pesticide, anti-androgenic flavor, hemolytic, 5-alpha reductase inhibitor (Omotoso *et al*., 2014; Hema *et al*., 2011). Previous studies suggest that trypanosomes are more susceptible to cellular damages by activated oxygen species ($O_2$, OH, $H_2O_2$) than mammalian cells (Fairlamb, 1982). This forms the basis for the use of nifurtimox and haematoprophyrin as trypanocides (Docampo and Stoppani, 1979).

9,12-octadecadienoic methyl ester, phytol present in the fractions have anti-cancer activity (Omotoso *et al*., 2014, Hema *et al*., 2011). 9-octadecenoic acid however, possesses anti-tumour activity (Omotosho et al., 2014). Anti-tumour drugs are screened for trypanocidal action (Williamson and Scott-Finnigan, 1978). Trypanocidal drugs are also screened for anti-cancer activity (Barrett and Barrett, 2000, Ivan *et al*., 2014)). This is perhaps due to the fact that Hide, (1989) suggested that protozoan parasites, such as those of malaria, trypanosomiasis and leishmaniasis, have a number of features in common with the proliferating cells of cancer and some forms of heart diseases. They appear, for instance, to have molecules that function as uptake points (receptors) for essential nutrients and growth factors that they must acquire from their hosts if they are to survive and thrive. In addition, trypanosomes exhibit antigenic variation, and cancer cells also have the capacity to undergo antigenic modulation (Ouaissi and Ouaissi, 2005). DL-alpha-difluoromethylornithine (DFMO) originally developed for use against cancer has been found to be an effective inhibitor of polyamine synthesis by inhibiting ornithine decarboxylase, an enzyme essential for the growth and multiplication of trypanosomes (Bacchi *et al*., 1982; McCann *et al*., 1983).

Anti-inflammatory activity has been ascribed to 9-octadecenoic acid, phytol and 9, 12-octadecadienoic acid. Trypanosomiasis gives rise to the development of inflammatory responses that contribute to the development of inflammation associated with tissue injury. The production of excessive inflammatory cytokines has been implicated in the induction of infection-associated pathogenicity (Mekata *et al*., 2012).

9,12 -octadecadienoic acid methyl ester is associated with insectifuge activity which can be harnessed as repellent against the tsetse fly, vector of trypanosomes. Avoidance of host-vector has been recommended as a method of choice for the control of vector borne diseases (WHO, 2015).

9-Octadecenamide has been used for the treatment of mood and sleep disorders as well as cannabinoid-regulated depression and for the treatment of atherosclerosis (Mechoulam *et al*., 1997).

In the second stage of trypanosomiasis infection, the parasites cross the blood-brain barrier to infect the central nervous system; the neurological or meningo-encephalic stage. Changes of behaviour, confusion, sensory disturbances, poor co-ordination, and disturbance of the sleep cycle are typical of the disease (WHO, 2015). Thus the use of the plant material studied in this work would be of some importance in

late stage treatment of Human African Trypanosomiasis.

It is obvious that the plant has gained popularity in Nigeria to be widely used as traditional medicine justify by the presence of Phytol. For it is use as a precursor for the manufacturing of synthetic form of vitamins E and $K_1$ that protect animals against status epilepticus induced pilocarpine and decreased the mortality rate (Costa *et al.* 2012) and part of chlorophyll (Vetter *et al.,* 2012).

A growing evidence have shown to indicate that octadecanamide mediate fundamental neurochemical process including sleep thermoregulation, nociception, prostaglandins and other lipids (Chaturvedi *et al.,* 2006). 9,12-octadecadienoic acid (antibacterial), octadecanoic acid (antimicrobial, hardener and thickener use as skin cleaner in soap industries) 9,12,15 octadecatrienoic acid methyl ester for antibacterial, anticandidal, antiinflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge antihistaminic, antiarthritic, anticoronary, antieczemic antiacne, 5-alpha reductase inhibitor antiandrogenic and 2(4H)-Benzofuran-5,6,7,7a-tetrahydro-4,4,7a-trimethyl for antimicrobial (Mujeeb, 2014). 2,2,4,4-tetrametyl-1,3-cyclobutanedione is well known building block for the sterically congested system (Brunck, 2001). These compounds are synergiscally responsible for the activities of the plant which can be harness for the development of our developing countries in the area of pharmacological techniques and economic improvement.

**Acknowledgements**

Authors acknowledge the support of the technologists in Nigeria Institute of Trypanosomiasis Research Laboratory, Vom and to the technical staff in Federal School Forestry, Jos for their various efforst they have put in to bring out the research study to this conclusion.

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4/22/2017