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## Characterization of Bioactive Chemical Compounds from *Aspergillus terreus* and Evaluation of Antibacterial and Antifungal Activity

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### ABSTRACT

The aim of this research was analysis of the bioactive chemical products and evaluation of antibacterial and antifungal activity of *Aspergillus terreus*. Bioactives (chemical compounds often referred to as secondary metabolites) were analyzed using gas chromatography-mass spectroscopy (GC-MS) technique, then the in vitro antibacterial and antifungal activity of the methanolic extract was evaluated. Forty seven bioactive compounds were identified in the methanolic extract of *Aspergillus terreus*. Crude extract of *Gramineae poaceae* plant was very highly active ( $7.47 \pm 0.14$ ) mm. The results of antibacterial activity produced by *Aspergillus terreus* showed that the volatile compounds were highly effective to suppress the growth of *Streptococcus pneumonia*. *Aspergillus terreus* produce many important secondary metabolites with high biological activities. Based on the significance of employing bioactive compounds in pharmacy to produce drugs for the treatment of many diseases, the purification of compounds produced by *Aspergillus terreus* species can be useful.

**Keywords:** *Aspergillus terreus*, Antibacterial activity, Antifungal activity, GC/MS, Secondary metabolites.

### INTRODUCTION

Secondary metabolites is a prerequisite for the development of novel pharmaceuticals and this is an especially urgent task in the case of antibiotics due to the rapid spreading of bacterial resistance and the emergence of multiresistant pathogenic strains, which severe clinical problems in the treatment of infectious disease. Many fungi are parasites on plants, animals (including humans), and even other fungi. The organism most often used for Itaconic acid (IA) production is *Aspergillus terreus*, grown under phosphate-limited conditions<sup>1-3</sup>, although some species of the plant pathogenic fungal genus *Ustilago*, a basidiomycete, are also known to produce IA during fermentation. Many fungi are parasites on plants, animals (including humans), and even other fungi. Some fungi can cause serious diseases in humans, several of which may be fatal if untreated. These include aspergilloses, candidoses and coccidioidomycosis<sup>4-8</sup>. The pharmaceutical industry has become increasingly interested in screening fungi for novel antibiotics and other secondary metabolites. Many fungi produce biologically active compounds, several of which are toxic to animals or plants. These are called mycotoxins. Particularly relevant to humans are mycotoxins produced by molds which cause food spoilage<sup>9-10</sup>. New secondary metabolites available from microorganisms may be used to optimize their availability by fermentation for further research and also for production in the pharmaceutical industry. The objective of this study was screening of secondary metabolites and evaluation of antimicrobial activity.

### MATERIALS AND METHODS

#### *Growth conditions and determination of metabolites*

*Aspergillus terreus* was isolated from dried fruit and the pure colonies were selected, isolated and maintained in potato dextrose agar slants<sup>11-12</sup>. Spores were grown in a liquid culture of potato dextrose broth (PDB) and incubated at 25°C in a shaker for 16 days at 130 rpm. The extraction was performed by adding 25 ml methanol to 100 ml liquid culture in an Erlenmeyer flask after the infiltration of the culture. The mixture was incubated at 4°C for 10 min and then shook for 10 min at 130 rpm. Metabolites was separated from the liquid culture and evaporated to dryness with a rotary evaporator at 45°C. The residue was dissolved in 1 ml methanol, filtered through a 0.2 µm syringe filter, and stored at 4°C for 24 h before being used for GC-MS. The identification of the components was based on comparison of their mass spectra with those of NIST mass spectral library as well as on comparison of their retention indices either with those of authentic compounds or with literature values<sup>13-14</sup>.

#### *Spectral analysis of bioactive chemical compounds using gas chromatography-mass spectrometry (GC/MS)*

Analysis was conducted using GC-MS (Agilent 789 A) equipped with a DB-5MS column (30 m×0.25 mm i.d., 0.25 µm film thickness, J&W Scientific, Folsom, CA). The oven temperature was programmed as for the previous analysis. Helium was used as the carrier gas at the rate of 1.0 mL/min. Effluent of the GC column was introduced directly into the source of the MS via a transfer line (250°C). Ionization voltage was 70 eV and ion source temperature was 230°C. Scan range was 41- 450 amu. The

Table 1: Major bioactive chemical compounds identified in methanolic extract of *Aspergillus terreus*.

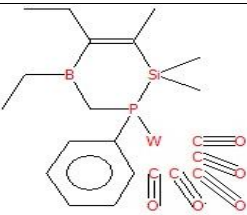
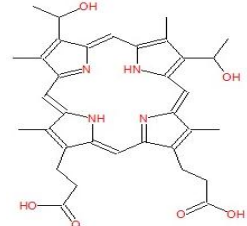
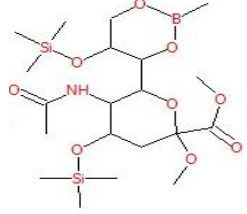
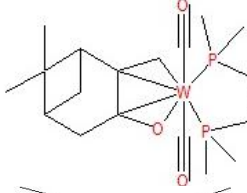
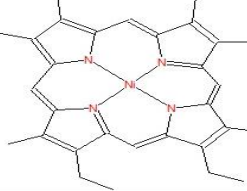
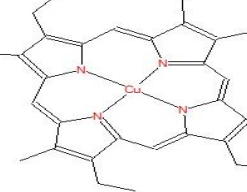
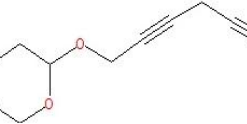
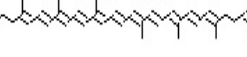
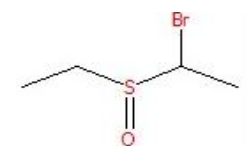
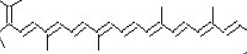
S No.	Phyto-chemical compound	RT (min)	Mol. Wt.	Exact Mass	Chemical structure	MS Fragment-ions
1.	Tungsten, pentacarbonyl(4,5-diethyl-2,2,2-trimethyl-1-phenyl-1-	3.144	612	612.088947		59,73,97,13 5,292,383,4 68,498,526, 556
2.	Hematoporphyrin	3.224	598	598.279137		53,67,108,2 37,450
3.	$\beta$ -N-Acetylneuraminic acid, methyl ester-2-methyl-7,9-methyl-bor	3.276	505	505.233465		59,75,103,1 39,196,227, 259,298,34 3,387,446
4.	Tungsten,dicarbonyl-(n-4-pinocarvone)[1,2-bis(dimethylphosphi	3.333	540	540.11795		53,69,108,1 35,240,287, 332390,438 ,,482,540
5.	Nickel,[2,8,12,18-tetraethyl-3,7,13,17-tetramethyl-21H,23H-por	3.356	534	534.22934		108,230,26 7,534
6.	Copper,[2,8,12,18-tetraethyl-3,7,13,17-tetramethyl-21H,23H-po	3.407	539	539.223595		108,207,27 0,332,402,5 39
7.	2H-Pyran,2-(2,5hexadiynyloxy)tetrahydro-	3.465	178	178.09938		51,65,77,14 1,170
8.	Lycoxanthin	3.516	552	552.433117		55,91,119,1 71,331,428, 534
9.	1-Bromo-ethanesulfinyl)-ethane	3.539	183	183.955748		50,63,78,10 7,184
10.	$\beta$ Carotene	3.648	536	536.4382		55,69,105,1 45,197,268,

Table 1: Major bioactive chemical compounds identified in methanolic extract of *Aspergillus terreus*.

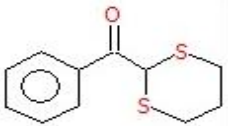
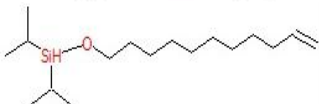
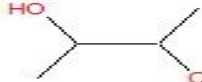

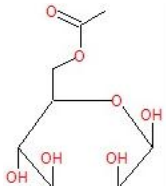
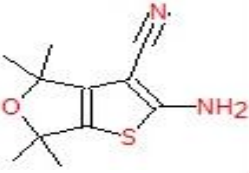

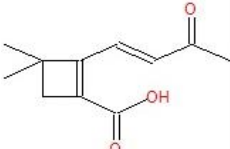
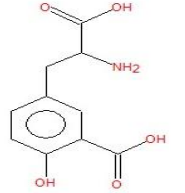
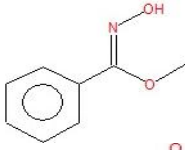
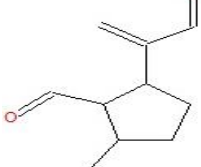
S No.	Phyto-chemical compound	RT (min)	Mol. Wt.	Exact Mass	Chemical structure	MS Fragment-ions
11.	Methanone ,1,3dithian-2-ylphenyl-	3.676	224	224.032957		378,397,44 4,536 51,65,69,75 ,77,81,85,9 1,105,119
12.	1-Diisopropylsilyloxy-10-undecene	3.728	284	284.253542		61,75,89,21 3,241
13.	2-Butanol , 3-chloro-, (R*,R*)-	3.808	108	108.0341926		50,55,63,73 ,79,85,93,1 05
14.	8,11-Octadecadiynoic acid , methyl ester	3.859	290	290.22458		55,78,91,10 5,119,133,1 45,162,173, 205,233
15.	6-Acetyl-β-d-mannose	4.203	222	222.073953		60,81,97,10 9,126,144,1 92
16.	Thieno[2,3-c]furan-3-carbonitrile,2-amino-4,6-dihydro-4,4,6,6-tetra	4.306	222	222.0826845		60,77,96,12 1,165,207,2 22
17.	17-Octadecynoic acid	4.357	280	280.24023		55,67,81,95 ,109,123,13 7,163,185,2 06,233,261
18.	1-Butan-3-one,1-(2-carboxy-4,4-dimethylcyclobutenyl)-	4.437	194	194.094295		56,65,79,91 ,105,133,15 1,179,194
19.	3-(3-Carboxy-4-hydroxyphenyl)-D-alanine	4.649	225	225.063723		51,77,106,1 33,151,208, 222
20.	Oxime-, methoxy-phenyl-	4.678	151	151.063329		55,73,105,1 33,151
21.	Cyclopentaneacetaldehyde , 2-formyl-3-methyl-α-methylene-	4.838	166	166.09938		55,81,95,10 9,151,165

Table 1: Major bioactive chemical compounds identified in methanolic extract of *Aspergillus terreus*.

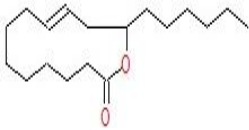
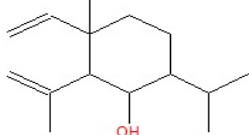
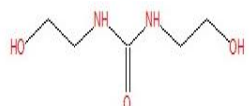
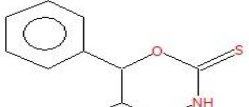
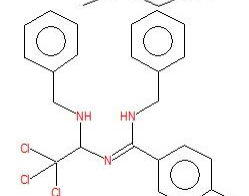
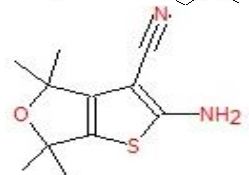
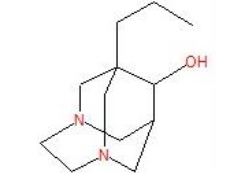
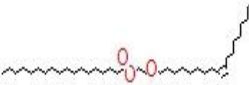
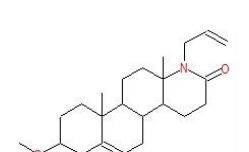
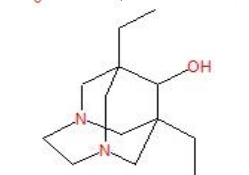
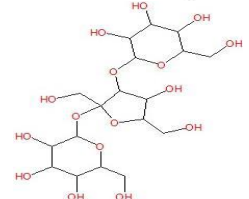
S No.	Phyto-chemical compound	RT (min)	Mol. Wt.	Exact Mass	Chemical structure	MS Fragment-ions
22.	13-Hexyloxacyclotridec-10-en-2-one	4.941	280	280.24023		55,67,81,98,109,137,151,166,182,210,262,280
23.	6-epi-shyobunol	4.992	222	222.198365		55,67,81,93,109,161,207,222
24.	Urea , N,N'-bis(2-hydroxyethyl)-	5.204	148	148.084792		61,81,132,146
25.	5-Methyl-6-phenyltetrahydro-1,3-oxazine-2-thione	5.175	207	207.071785		57,77,91,117,132,147,174,207
26.	N-Benzyl-N'-(1-benzylamino-2,2,2-trichloroethyl)-p-tolylcarboxam	5.152	459	459.10358		51,65,91,106,118,164,223
27.	Thieno[2,3-c]furan-3-carbonitrile,2-amino-4,6-dihydro-4,4,6,6-	5.313	222	222.0826845		60,96,121,165,207,222
28.	1-Propyl-3,6-diazahomoadamantan-9-ol	5.387	210	210.173213		58,72,82,136,181,193,210
29.	Stearic acid , 2-(9-octadecenyl-oxy)ethyl ester . (Z)-	5.467	578	578.563797		57,69,83,123,207,250,267,281,311,329
30.	17a-Allyl-3β-methoxy-17a-aza-D-homoandrost-5-ene-17-one	5.599	357	357.266779		55,71,91,105,150,205,253,310,342,357
31.	1,8-Diethyl-3,6-diazahomoadamantan-9-ol	5.708	224	224.188864		58,72,96,110,136,152,166,180,195,224
32.	α-D-Glucopyranoside,O-α-D-glucopyranosyl-(1.fwdarw.3)-β-	7.373	504	504.169035		60,73,85,97,113,126,145,191

Table 1: Major bioactive chemical compounds identified in methanolic extract of *Aspergillus terreus*.

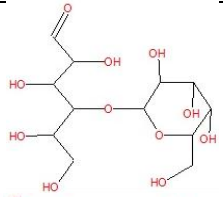
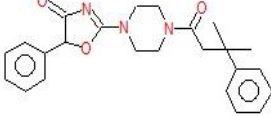
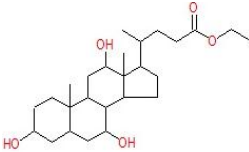
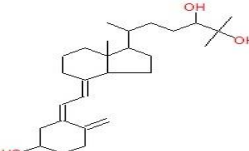
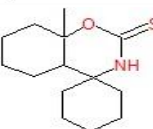
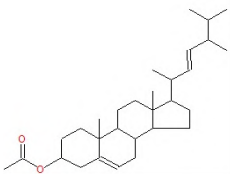
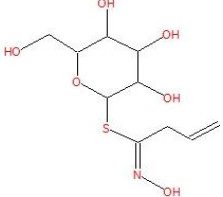
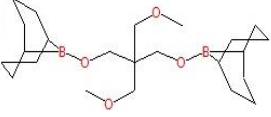
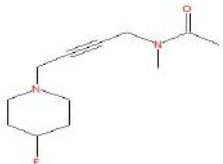
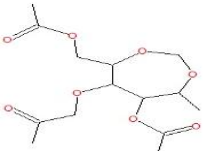
S No.	Phyto-chemical compound	RT (min)	Mol. Wt.	Exact Mass	Chemical structure	MS Fragment-ions
33.	Lactose	7.710	342	342.11621		60,73,97,12 6,163,207
34.	1-[[3-Methyl-3-phenyl]butyryl-4-[5-phenyl-4-oxo-2-oxazolin-2-yl]]	7.951	405	405.205242		56,69,91,10 5,119,153,1 77,202,245, 287,337,40 5
35.	Ethyl iso-allocholate	8.162	436	436.318874		55,69,81,95 ,145,213,25 3,400,418
36.	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol,(3β,5Z,7E)-	8.425	416	416.329004		55,69,91,11 8,136,158,1 76,189,207, 221,253,38 3,416
37.	5,6,7,8,9,10-Hexahydro-9-methyl-spiro[2H-1,3-benzoxazine]-	8.660	253	253.150035		55,69,81,97 ,158,176,22 0,253
38.	Ergosta-5,22-dien-3-ol,acetate,(3β,22E)-	8.820	440	440.36543		55,67,91,14 5,213,255,2 81,327,380
39.	Desulphosinigrin	9.455	279	279.077658		60,73,85,10 3,127,145,1 63,213,262
40.	Pentaerythritol,bis-O-(9-borabicyclo[3.3.1]non-9-yl)-di-O-methyl-	9.879	404	404.326921		55,67,85,10 9,127,143,1 81,223,258, 293,347,40 4
41.	Acetamide, N-methyl-N-[4-[4-fluoro-1-hexahydropyridyl]-2-butynyl]	11.252	226	226.148142		58,82,94,12 4,168,211
42.	1,4-Diacetyl-3-acetoxymethyl-2,5-methylene-1-rhamnitol	11.464	318	318.131468		57,103,115, 145,191,23 3,263,318

Table 1: Major bioactive chemical compounds identified in methanolic extract of *Aspergillus terreus*.

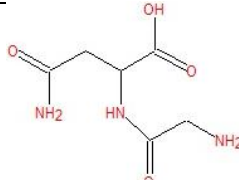
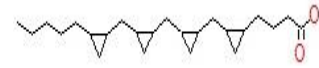
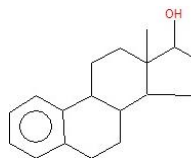
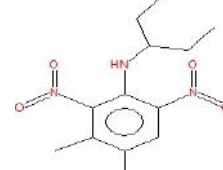
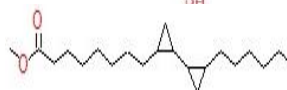
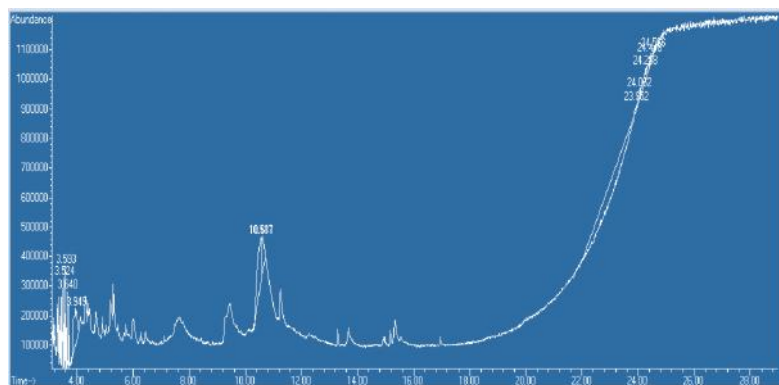
S No.	Phyto-chemical compound	RT (min)	Mol. Wt.	Exact Mass	Chemical structure	MS Fragment-ions
43.	Glycyl-D-asparagine	12.253	189	189.074956		55,72,100,113,154
44.	Cyclopropanebutanoic acid, 2-[[2-[[2-(2-pentylcyclopropyl)methyl	13.283	374	374.318481		55,74,95,121,135,149,161,199,227,270,298,334
45.	Estra-1,3,5(10)-trien-17β-ol	13.678	256	256.182714		57,73,85,97,129,157,185,213,241,256
46.	Benzenemethanol,4-[(ethylpropyl)amino]-2-methyl-3,5-dinitro-	14.708	297	297.132471		57,65,77,91,104,131,178,208,268,297
47.	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-,methyl ester	15.343	322	322.28718		55,73,81,95,109,123,137,165,192,224,291,322

Figure 1: Morphological characterization of *Aspergillus terreus* colonyFigure 2: GC-MS chromatogram of methanolic extract of *Aspergillus terreus*.

components were identified by comparing their retention times to those of authentic samples of WILEY MASS SPECTRAL DATA BASE Library<sup>15-16</sup>.

#### Determination of antibacterial and antifungal activity

*Streptococcus pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis* and *Staphylococcus epidermidis* were swabbed in Muller-Hinton agar plates. 90 µl of fungal extracts was loaded on the bored wells. The wells were bored in 0.5 cm in diameter. The plates were incubated at 37°C for 24 hrs and examined<sup>17</sup>. After the incubation the diameter of inhibition zones around the discs was measured. *Aspergillus terreus*

isolate was suspended in potato dextrose broth and diluted to approximately 105 colony forming unit (CFU) per ml. They were "flood inoculated onto the surface of Potato dextrose agar and then dried. Standard agar well diffusion method was followed<sup>18-20</sup>. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 25 µl of the samples solutions were delivered into the wells. The plates were incubated for 48 h at room temperature. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test microorganisms. Methanol was used as solvent control. Amphotericin B and fluconazole were used as reference antifungal agent<sup>21</sup>. The



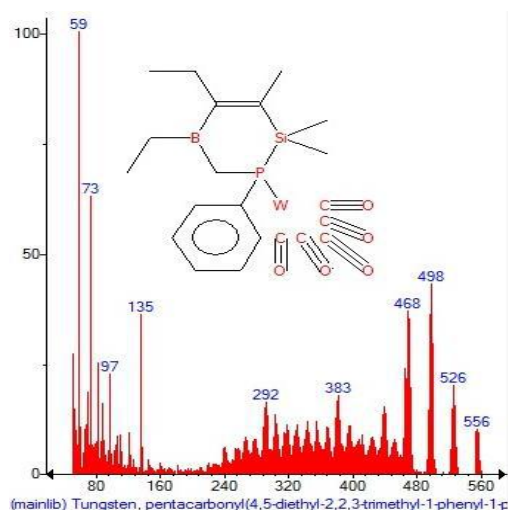


Figure 3: Mass spectrum of Tungsten, pentacarbonyl(4,5-diethyl-2,2,2-trimethyl-1-phenyl-1-p) with Retention Time (RT)= 3.144

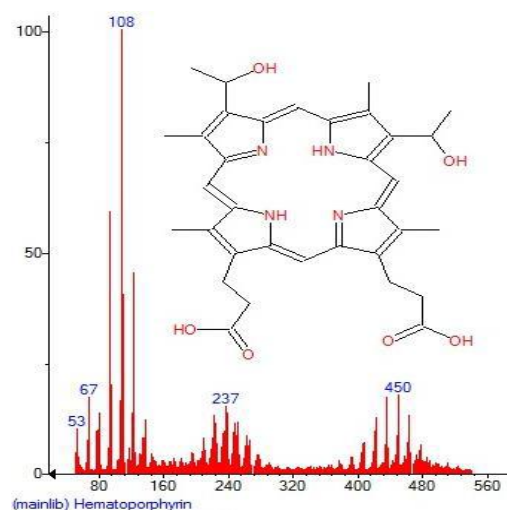


Figure 4: Mass spectrum of Hematoporphyrin with Retention Time (RT)= 3.224

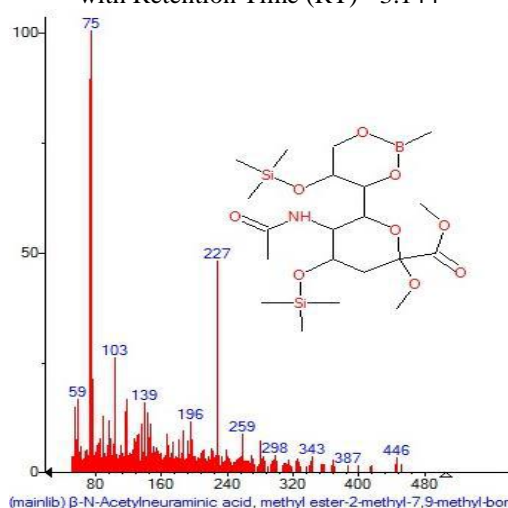


Figure 5: Mass spectrum of β-N-Acetylneuraminic acid, methyl ester-2-methyl-7,9-methyl-bor with Retention Time (RT)= 3.276

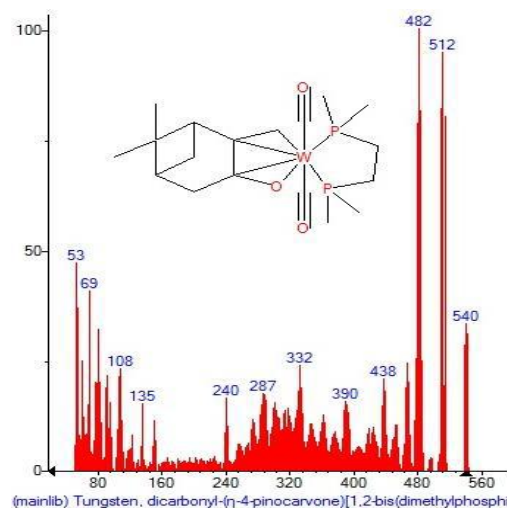


Figure 6: Mass spectrum of Tungsten, dicarbonyl-(n-4-pinocavone)[1,2-bis(dimethylphosphi with Retention Time (RT)= 3.333

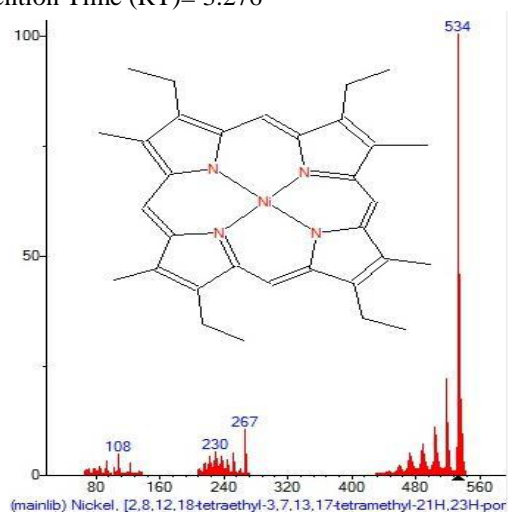


Figure 7: Mass spectrum of Nickel, [2,8,12,18-tetraethyl-3,7,13,17-tetramethyl-21H,23H-por with Retention Time (RT)= 3.356

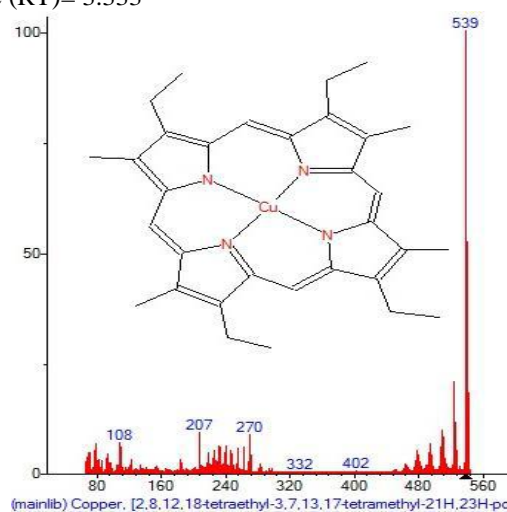


Figure 8: Mass spectrum of Copper, [2,8,12,18-tetraethyl-3,7,13,17-tetramethyl-21H,23H-po with Retention Time (RT)= 3.407



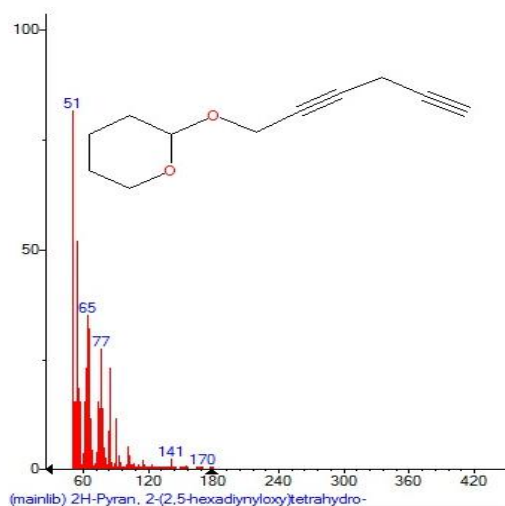


Figure 9: Mass spectrum of 2H-Pyran,2-(2,5hexadiynyloxy)tetrahydro- with Retention Time (RT)= 3.465

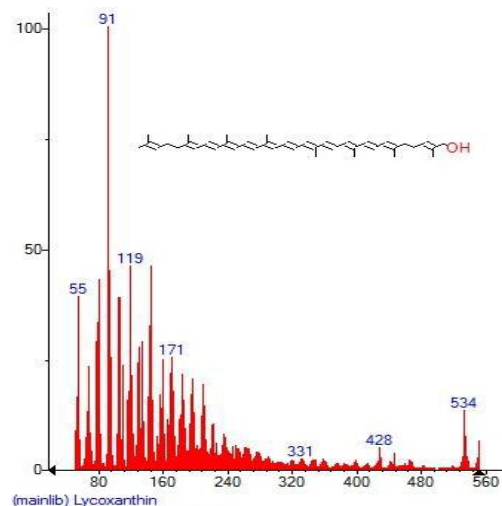


Figure 10: Mass spectrum of Lycoxanthin with Retention Time (RT)= 3.516

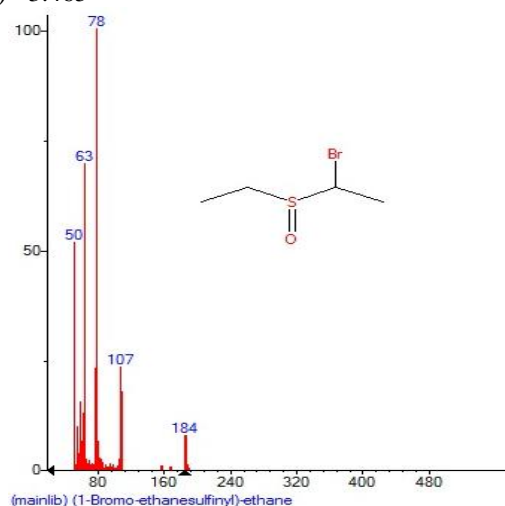


Figure 11: Mass spectrum of 1-Bromo-ethanesulfinyl)-ethane with Retention Time (RT)= 3.539

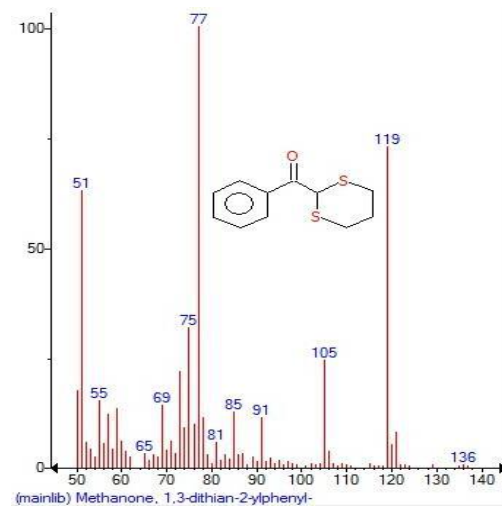


Figure 12: Mass spectrum of  $\beta$  Carotene with Retention Time (RT)= 3.648

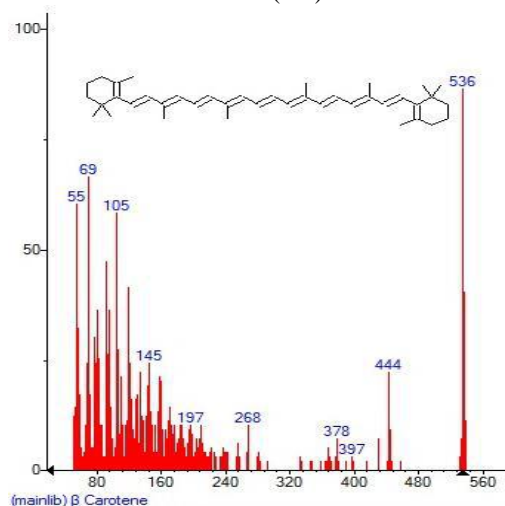


Figure 13: Mass spectrum of Methanone, 1,3dithian-2-ylphenyl- with Retention Time (RT)= 3.676

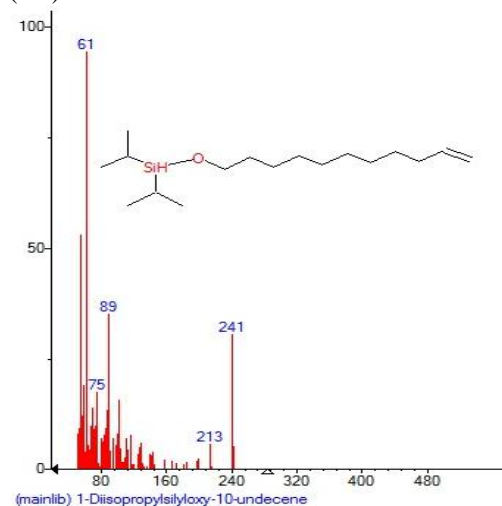


Figure 14: Mass spectrum of 1-Diisopropylsilyloxy-10-undecene with Retention Time (RT)= 3.728

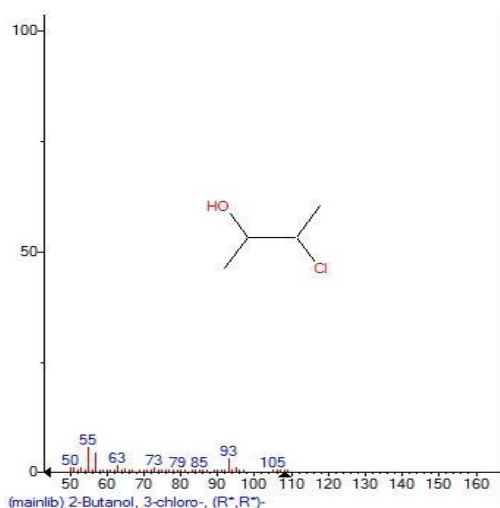


Figure 15: Mass spectrum of 2-Butanol, 3-chloro-, (R\*,R\*)- with Retention Time (RT)= 3.808

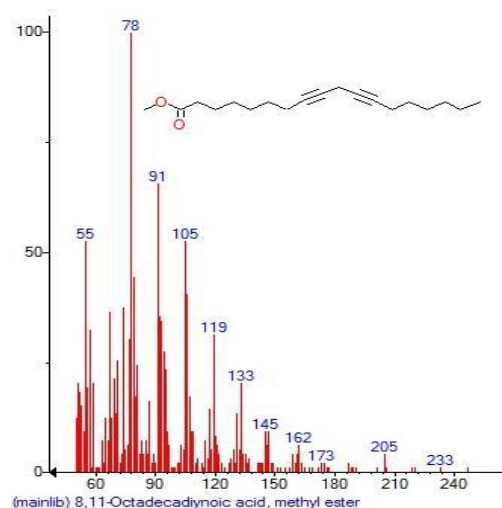


Figure 16: Mass spectrum of 8,11-Octadecadiynoic acid, methyl ester with Retention Time (RT)= 3.859

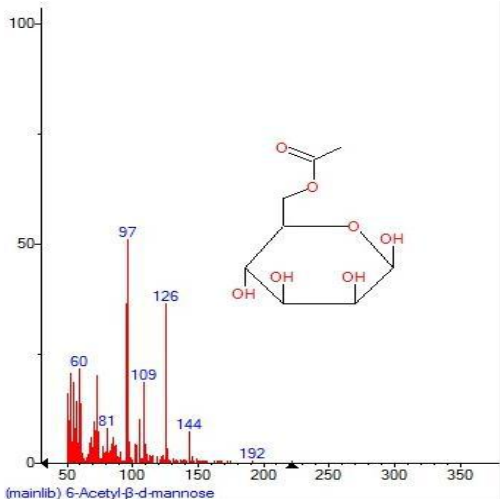


Figure 17: Mass spectrum of 6-Acetyl-beta-D-mannose with Retention Time (RT)= 4.203

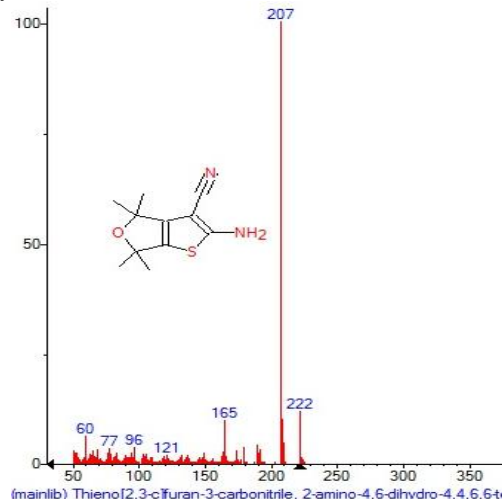


Figure 18: Mass spectrum of Thieno[2,3-c]furan-3-carbonitrile, 2-amino-4,6-dihydro-4,4,6,6-tetra with Retention Time (RT)= 4.306

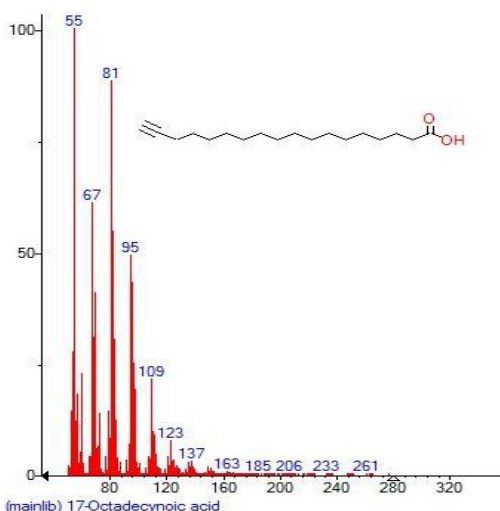


Figure 19: Mass spectrum of 17-Octadecynoic acid with Retention Time (RT)= 4.357

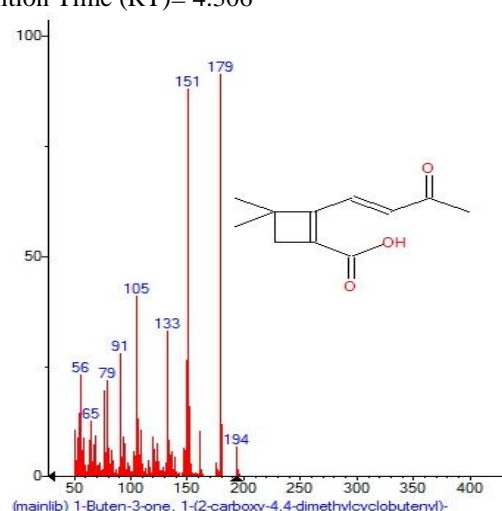


Figure 20: Mass spectrum of 1-Buten-3-one, 1-(2-carboxy-4,4-dimethylcyclobutenyl)- with Retention Time (RT)= 4.437

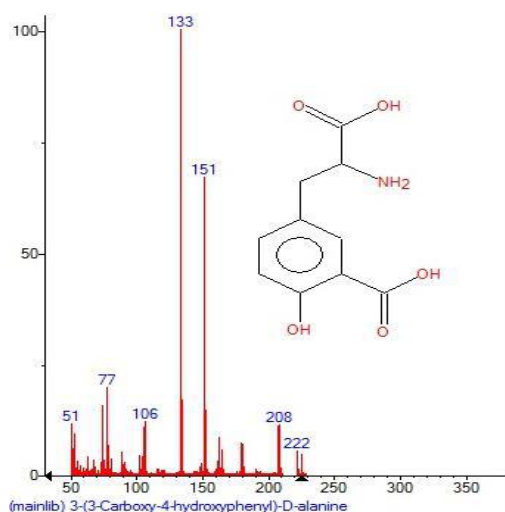


Figure 21: Mass spectrum of 3-(3-Carboxy-4-hydroxyphenyl)-D-alanine with Retention Time (RT)= 4.649

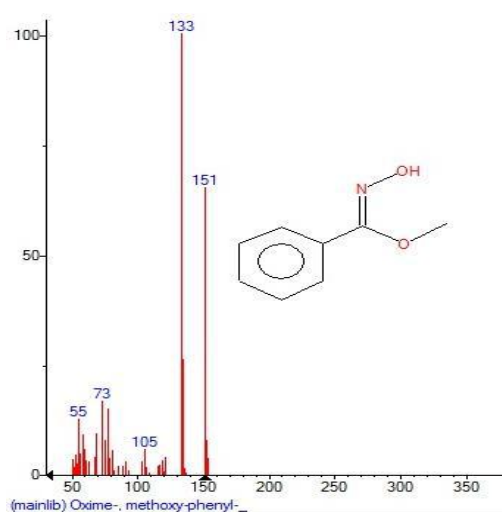


Figure 22: Mass spectrum of Oxime-, methoxy-phenyl- with Retention Time (RT)= 4.678

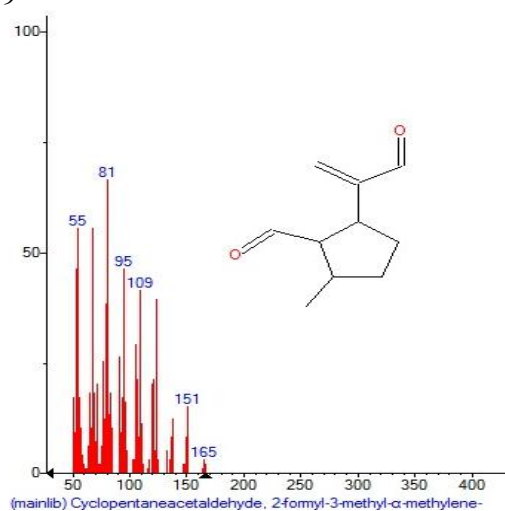


Figure 23: Mass spectrum of Cyclopentaneacetaldehyde, 2-formyl-3-methyl-α-methylene- with Retention Time (RT)= 4.838

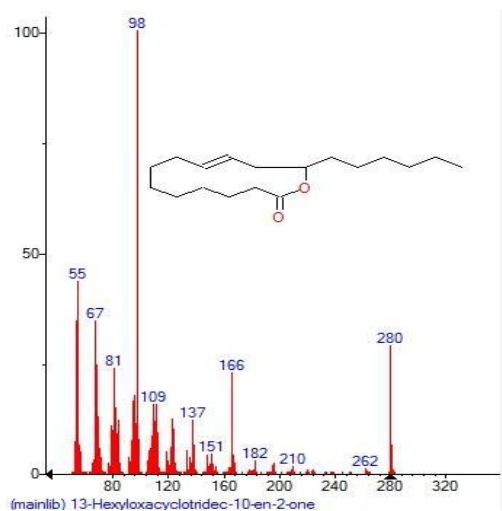


Figure 24: Mass spectrum of 13-Hexyloxacyclotridec-10-en-2-one with Retention Time (RT)= 4.941

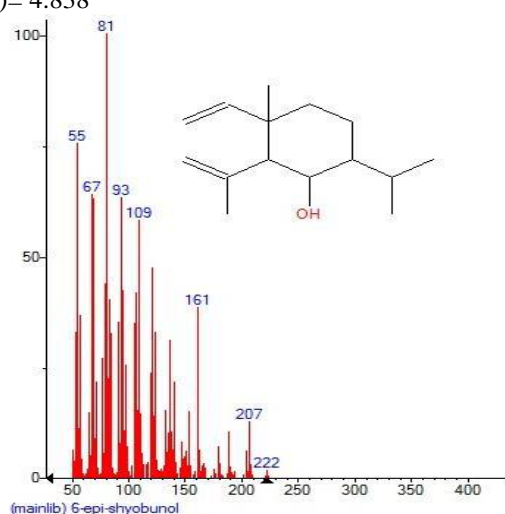


Figure 25: Mass spectrum of 6-epi-shyobunol with Retention Time (RT)= 4.992

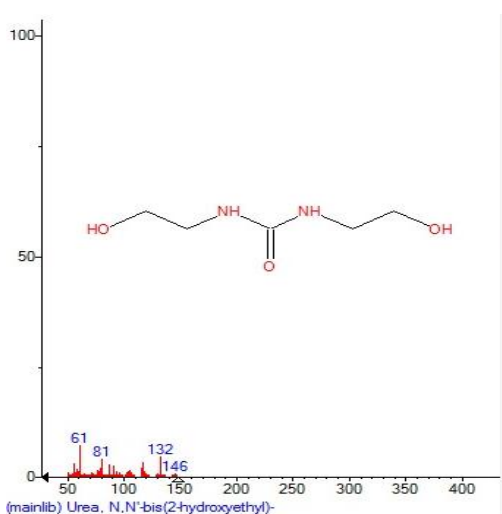


Figure 26: Mass spectrum of Urea, N,N'-bis(2-hydroxyethyl)- with Retention Time (RT)= 5.204

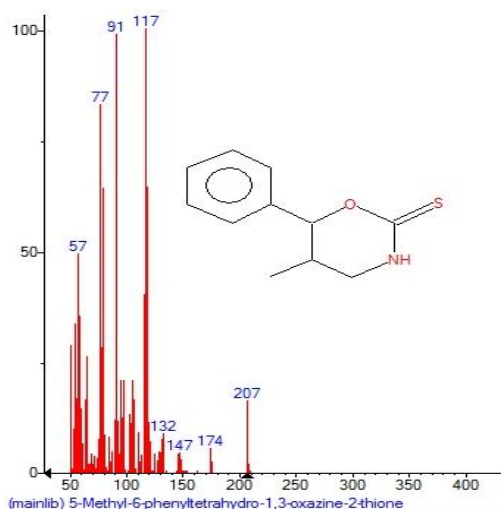


Figure 27: Mass spectrum of 5-Methyl-6-phenyltetrahydro-1,3-oxazine-2-thione with Retention Time (RT)= 5.175

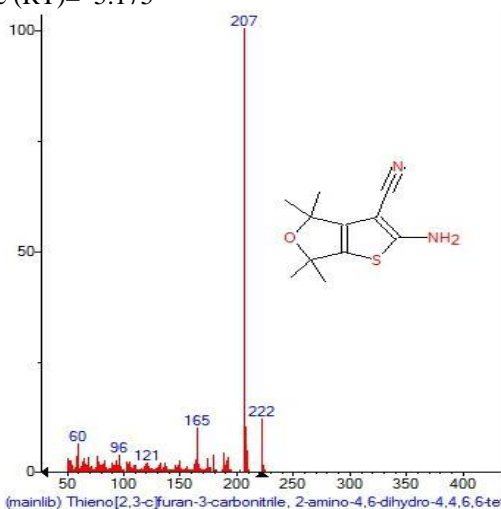


Figure 29: Mass spectrum of Thieno[2,3-c]furan-3-carbonitrile, 2-amino-4,6-dihydro-4,4,6,6-tetra-methyl- with Retention Time (RT)= 5.313

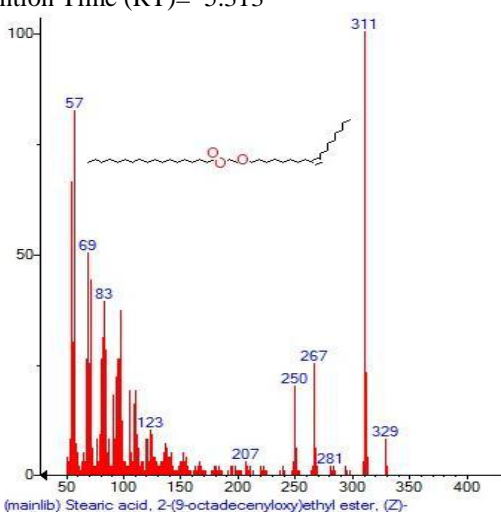


Figure 31: Mass spectrum of Stearic acid, 2-(9-octadecenyloxy)ethyl ester, (Z)- with Retention Time (RT)= 5.467

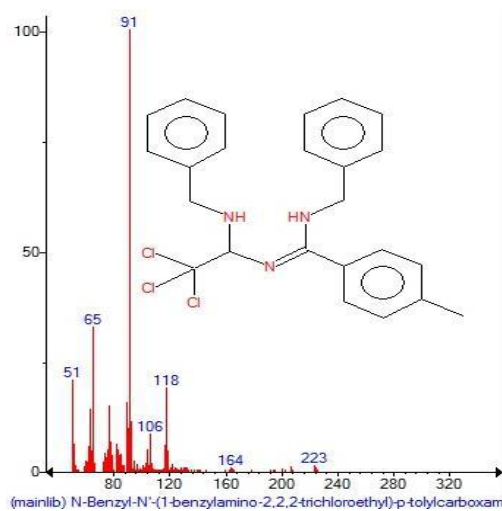


Figure 28: Mass spectrum of N-Benzyl-N'-(1-benzylamino-2,2,2-trichloroethyl)-p-tolylcarboxamide with Retention Time (RT)= 5.152

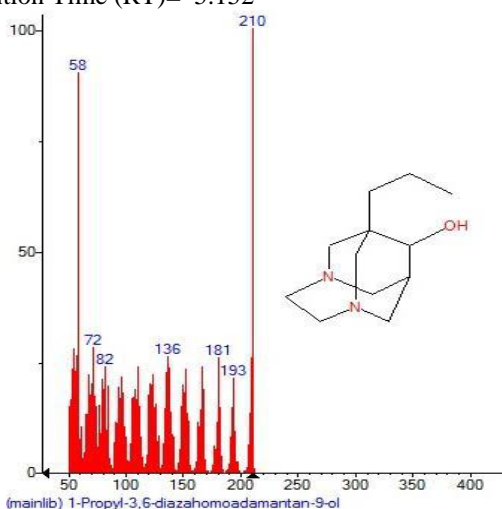


Figure 30: Mass spectrum of 1-Propyl-3,6-diazahomoadamantan-9-ol with Retention Time (RT)= 5.387

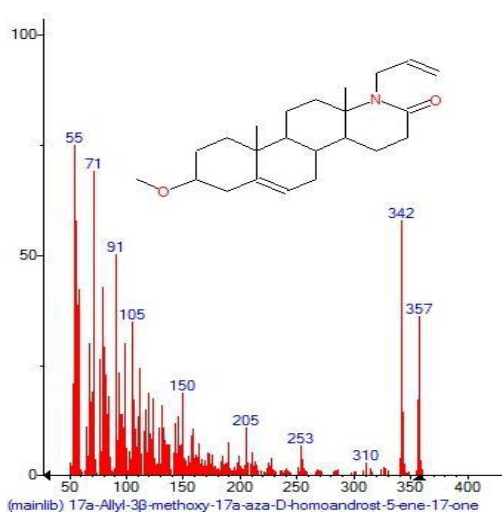


Figure 32: Mass spectrum of 17a-Allyl-3β-methoxy-17a-aza-D-homoandrost-5-ene-17-one with Retention Time (RT)= 5.599

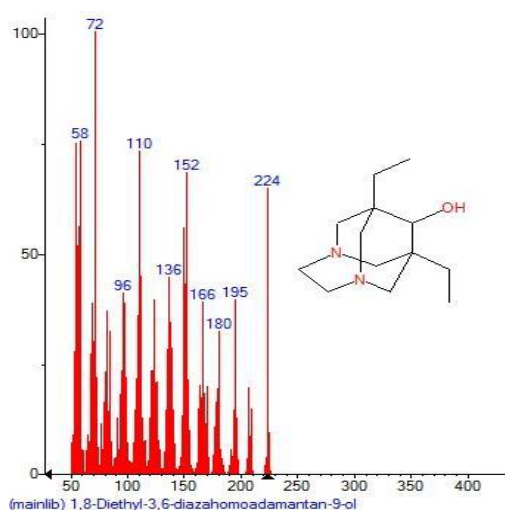


Figure 33: Mass spectrum of 1,8-Diethyl-3,6-diazahomoadamantan-9-ol with Retention Time (RT)= 5.708

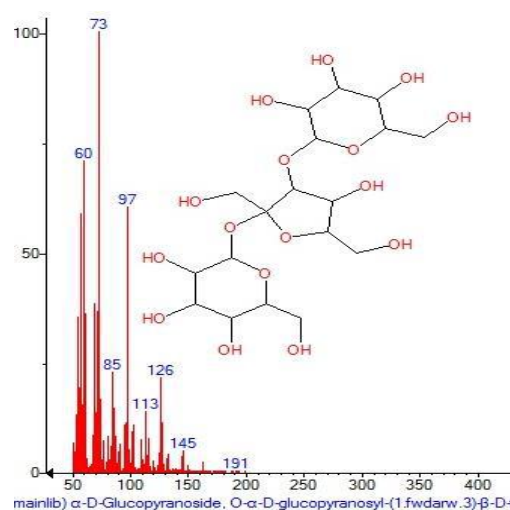


Figure 34: Mass spectrum of  $\alpha$ -D Glucopyranoside, O- $\alpha$ -D-glucopyranosyl-(1.fwdarw.3)- $\beta$ - with Retention Time (RT)= 7.373

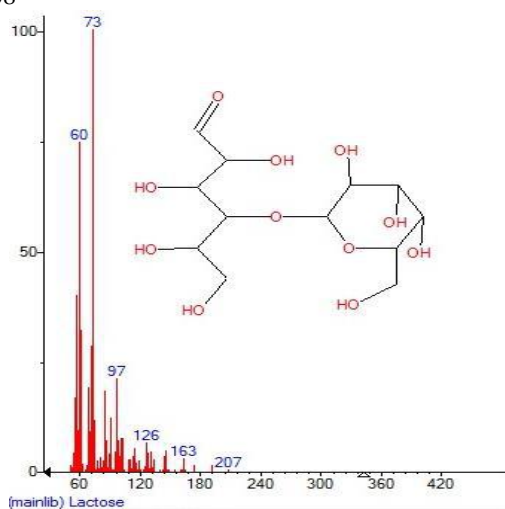


Figure 35: Mass spectrum of Lactose with Retention Time (RT)= 7.710

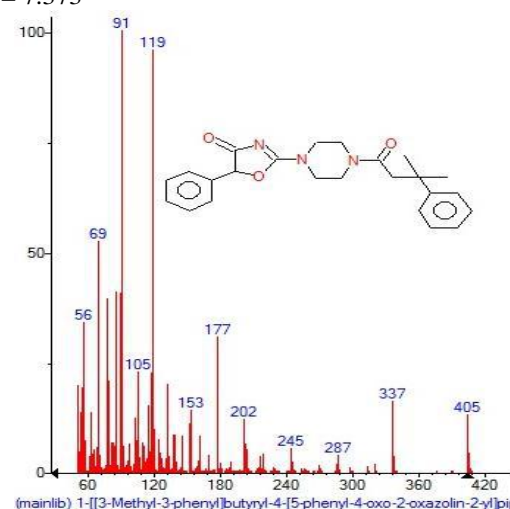


Figure 36: Mass spectrum of 1-[[3-Methyl-3-phenyl]butyryl-4-[5-phenyl-4-oxo-2-oxazolin-2-yl]]pi with Retention Time (RT)= 7.951

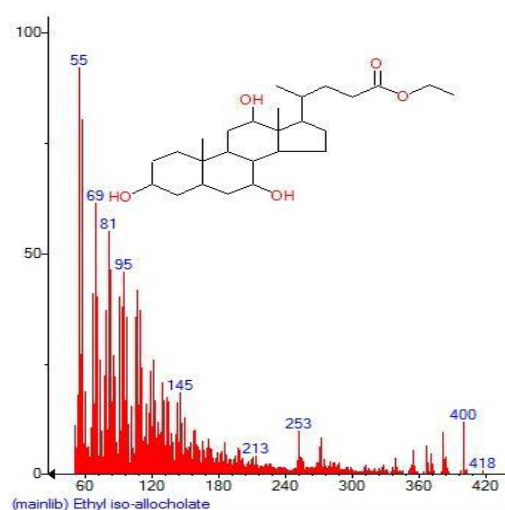


Figure 37: Mass spectrum of Ethyl iso-allocholate with Retention Time (RT)= 8.162

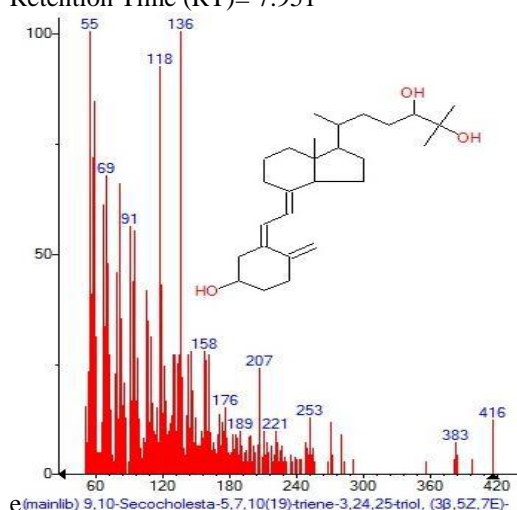


Figure 38: Mass spectrum of 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3 $\beta$ ,5Z,7E)- with Retention Time (RT)= 8.425



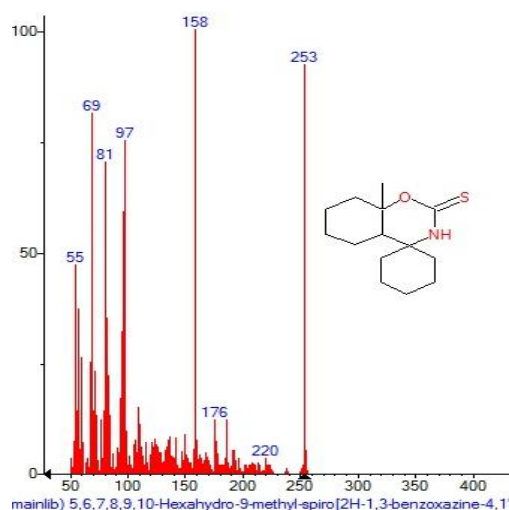


Figure 39: Mass spectrum of 5,6,7,8,9,10-Hexahydro-9-methyl-spiro[2H-1,3-benzoxazine- with Retention Time (RT)= 8.660

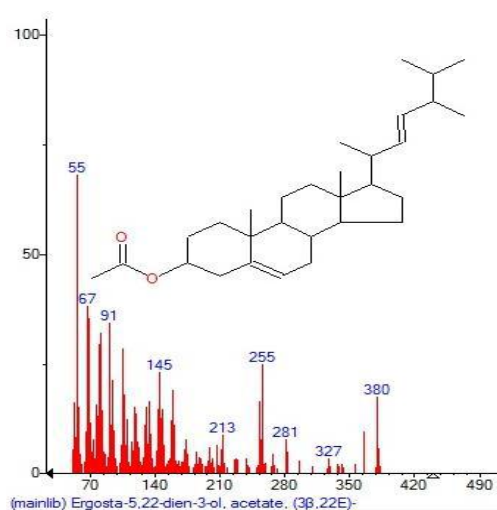


Figure 40: Mass spectrum of Ergosta-5,22-dien-3-ol,acetate,(3β,22E)- with Retention Time (RT)= 8.820

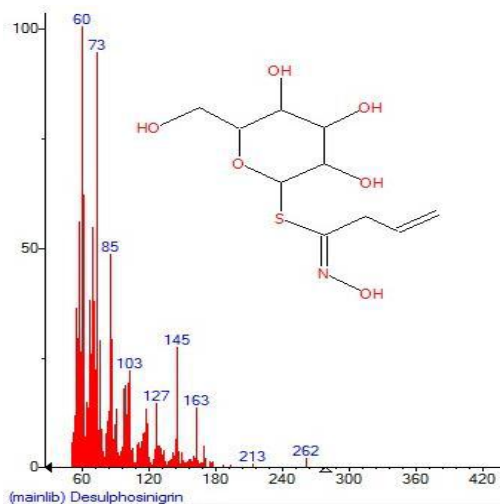


Figure 41: Mass spectrum of Desulphosinigrin with Retention Time (RT)= 9.455

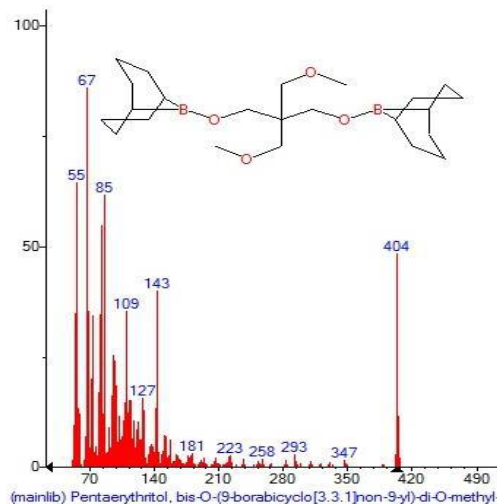


Figure 42: Mass spectrum of Pentaerythritol,bis-O-(9-borabicyclo[3.3.1]non-9-yl)-di-O-methyl- with Retention Time (RT)= 9.879

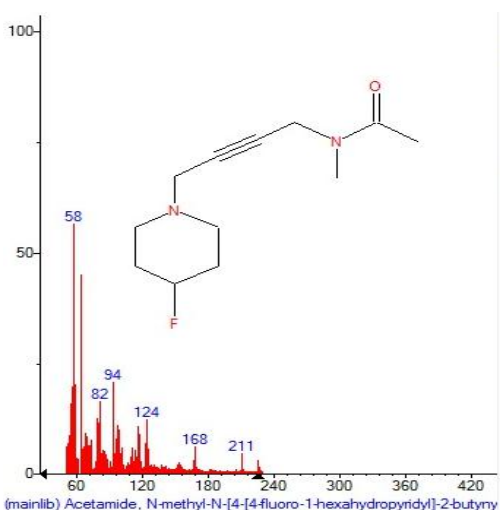


Figure 43: Mass spectrum of Acetamide, N-methyl-N-[4-[4-fluoro-1-hexahydropyridyl]-2-butynyl with Retention Time (RT)= 11.252

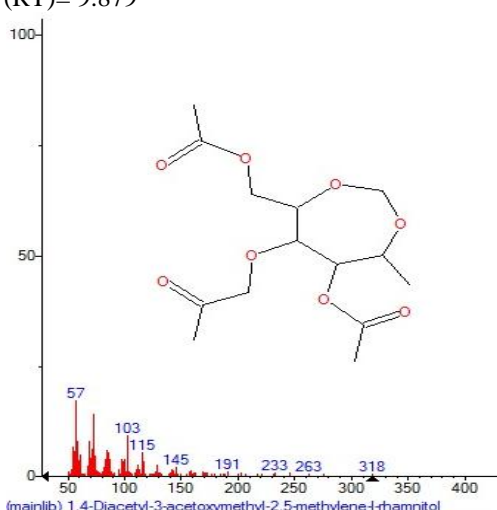


Figure 44: Mass spectrum of 1,4-Diacetyl-3-acetoxymethyl-2,5-methylene-1-rhamnitol with Retention Time (RT)= 11.464



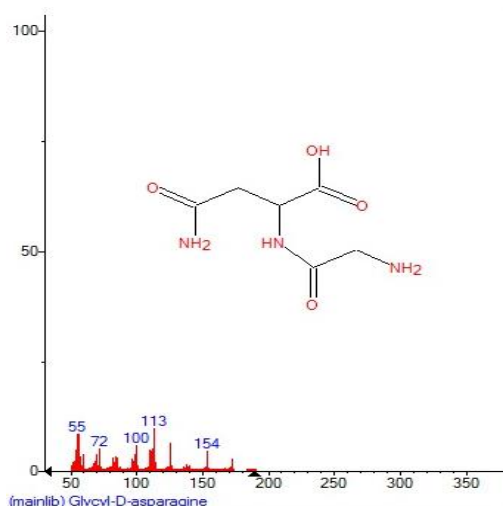


Figure 45: Mass spectrum of Glycyl-D-asparagine with Retention Time (RT)= 12.253

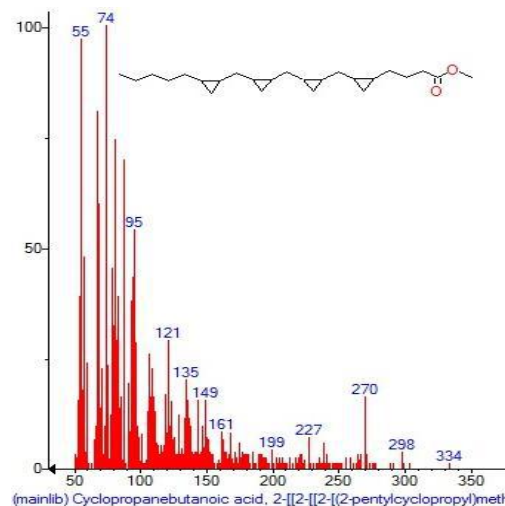


Figure 46: Mass spectrum of Cyclopropanebutanoic acid, 2-[[2-[[2-(2-pentylcyclopropyl)methyl]] with Retention Time (RT)= 13.283

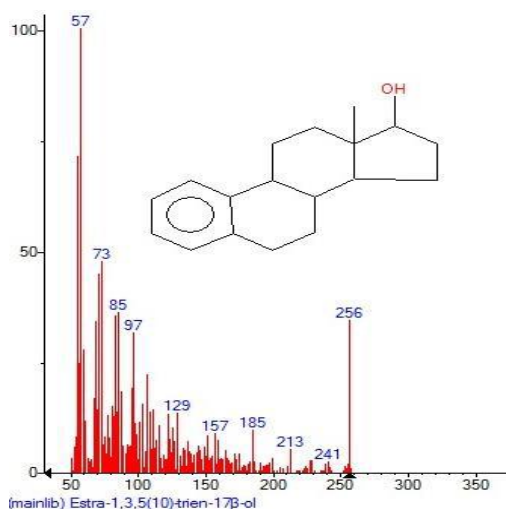


Figure 47: Mass spectrum of Estradiol with Retention Time (RT)= 13.678

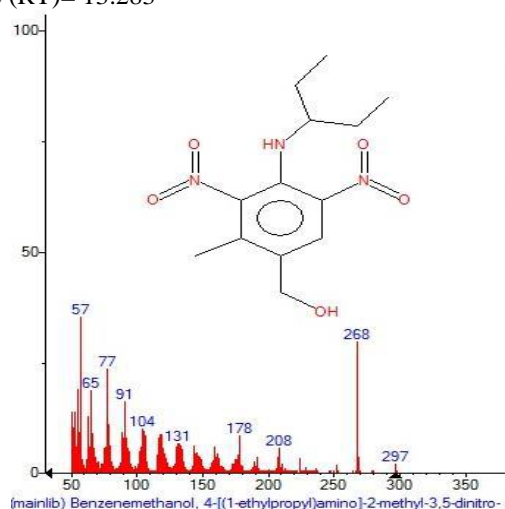


Figure 48: Mass spectrum of Benzenemethanol,4-[(ethylpropyl)amino]-2-methyl-3,5-dinitro- with Retention Time (RT)= 14.708

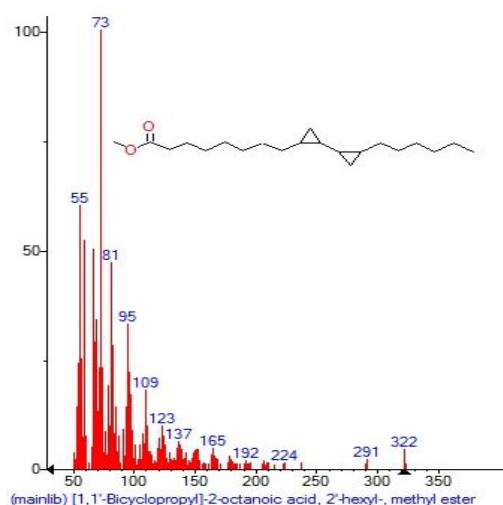


Figure 49: Mass spectrum of [1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-,methyl ester with Retention Time (RT)= 15.343

tests were carried out in triplicate. The antifungal activity was evaluated by measuring the inhibition-zone diameter observed after 48 h of incubation. Data were analyzed using analysis of variance (ANOVA) and differences among the means were determined for significance at  $P < 0.05$  using Duncan's multiple range test (by SPSS software) Version 9.1

## RESULTS AND DISCUSSION

Morphological, Microscopical and microscopical characteristics of fungal strains were determined using specific media light and compound microscope (fig. 1). The 400ml of fermentation broth (PDA broth) which contain 200μl of the standardized fugal suspensions were used to inoculate the flasks and incubated at 37°C on a shaker at 90 rpm for 7 days. After fermentation, the secondary metabolites were produced by isolated microorganisms. Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic extract of *Aspergillus terreus*, shown in (table 1). The GC-MS chromatogram of the thirty one peaks of

Table 2: Bioactivity of the methanolic crude extract of *Aspergillus terreus* and standard antibiotics against the five tested pathogens.

Fungal products Antibiotics	Zone of inhibition (mm)				
	Bacteria				
	Streptococcus pneumonia	Escherichia coli	Staphylococcus aureus	Proteus mirabilis	Staphylococcus epidermidis
Fungal products	6.32±0.013	2.99±0.300	6.09±0.200	6.22±0.210	5.00±0.203
Streptomycin	1.08±0.200	0.97±0.116	2.08±0.233	3.04±0.261	0.98±0.166
Kanamycin	1.02±0.180	1.00±0.190	2.08±0.236	1.00±0.100	1.82±0.200
Rifampin	2.00±0.202	2.00±0.103	1.99±0.182	0.964±0.233	2.08±0.240
Cefotaxime	0.97±0.211	1.51±0.270	1.94±0.100	1.08±0.272	2.08±0.290

Table 3: Zone of inhibition (mm) of test different bioactive compounds and standard antibiotics of plants to *Aspergillus terreus*.

S. No.	Plant	Zone of inhibition (mm)
1.	Piper nigrum (Crude)	6.11±0.20
2.	Zingiber officinale (Crude)	5.00±0.22
3.	Gramineae poaceae (Crude)	7.97±0.14
4.	Nerium olender (Alkaloids)	4.21±0.21
5.	Ricinus communis (Alkaloids)	2.94±0.20
6.	Datura stramonium (Alkaloids)	3.56±0.21
7.	Linum usitatissimum (Crude)	4.97±0.15
8.	Anastatica hierochuntica (Crude)	5.11±0.19
9.	Linum usitatissimum (Crude)	4.37±0.14
10.	Cassia angustifolia (Crude)	6.35±0.26
11.	Euphorbia lathyrus (Crude)	5.77±0.28
12.	Rosmarinus officinalis (Crude)	5.48±0.16
13.	Mentha viridis (Crude)	5.59±0.15
14.	Artemisia annua (Crude)	5.73±0.29
15.	Quercus infectoria (Crude)	6.79±0.11
16.	Citrullus colocynthis (Crude)	4.21±0.21
17.	Althaea rosea (Crude)	5.99±0.16
18.	Coriandrum sativum (Crude)	5.82±0.19
19.	Melia azedarach (Crude)	3.98±0.26
20.	Origanum vulgare (Crude)	6.09±0.29
21.	Urtica dioica (Crude)	3.78±0.15
22.	Equisetum arvense (Crude)	5.69±0.14
23.	Foeniculum vulgare (Crude)	3.72±0.19
24.	Nigella sativa (Crude)	4.55±0.35
25.	Ocimum basilicum (Crude)	4.67±0.19
26.	Punica granatum (Crude)	6.00±0.25
27.	Cinnamomum zeylanicum (Crude)	4.72±0.20
28.	Amphotericin B	7.55±0.24
29.	Fluconazol	7.98±0.16
30.	Control	0.00

the compounds detected was shown in (fig. 2). The First set up peak were determined to be 1,2-cis-1,5-trans-2,5-dihydroxy-4-methyl-1-(1-hydroxy-1-isopropyl) cy, (fig. 3). The second peak indicated to be 2-Furancarboxaldehyde, 5-methyl, (fig. 4). The next peaks considered to be 2(5H)-Furanone, 6-Hydroxymethyl-5-methyl-bicyclo[3.1.0]hexan-2-one, D-Glucose, 6-O- $\alpha$ -D-galactopyranosyl, 2-(3-Hydroxy-propyl)-cyclohexane-1,3-dione, 9-Oxa-bicyclo[3.3.1]nonane-1,4-diol,

Benzenemethanol, 2-(2-aminopropoxy)-3-methyl, 1,2-Cyclopentanedione, 3-methyl,  $\alpha$ -D-Glucopyranoside, O- $\alpha$ -D-glucopyranosyl-(1-fwdarw.3)- $\beta$ -D-fruc, 1-Nitro-2-acetamido-1,2-dideoxy-d-mannitol, Desulphosinigrin, Orcinol, Bicyclo[2.2.1]heptane-2-carboxylic acid isobutyl-amide, 2H-Oxecin-2-one. 3.4.7.8.9.10-hexahydro-4-hydroxy-10-methyl-. [4, 2H-Pyran, tetrahydro-2-(12-pentadecynyloxy), Maltol, 2-Tridecyl-5-(acetylaminotetrahydro- $\gamma$ -pyrone, Cycloundecanone, oxime, D-Glucose, 6-O- $\alpha$ -D-galactopyranosyl, 6-Acetyl- $\beta$ -d-mannose, 5-Hydroxymethylfurfural, 1-Gala-1-ido-octonic lactone, Pterin-6-carboxylic acid, Uric acid, Acetamide, N-methyl-N-[4-[2-acetoxymethyl-1-pyrrolidyl]-2-butynyl], 1-(+)-Ascorbic acid 2,6-dihexadecanoate, D-fructose, diethyl mercaptal, pentaacetate, 2-Bromotetradecanoic acid, Octadecanal, 2-bromo, L-Ascorbic acid, 6-octadecanoate, 18,19-Secoyohimban-19-oic acid, 16,17,20,21-tetradecahydro-16. (fig. 5-49). Many compounds are identified in the present study. Some of them are biological compounds with antimicrobial activities. Many fungi are parasites on plants, animals (including humans)<sup>4</sup>. We have previously characterized the same in *Aspergillus niger*<sup>21</sup> and *Penicillium expansum* fungi<sup>10</sup>. Some fungi can cause serious diseases in humans, several of which may be fatal if untreated<sup>22</sup>. Fungal spores also cause allergies, and can evoke allergic reactions<sup>23</sup>. The attention of natural product chemists and pharmaceutical companies, at present, is focused firmly on anticancer drugs, with several promising sponge-derived substances in clinical and preclinical trials. Cultivation of sponge-associated microorganisms that produce bioactive substances is the most direct method for large-scale production of these chemicals<sup>24,25</sup>. As has been previously mentioned many fungi produce biologically active compounds, several of which are toxic to animals or plants<sup>26</sup>. Since growth under different culture conditions may influence which metabolites are produced, the use of many different media and conditions should help to maximize the chemical diversity from a given microorganism<sup>27-37</sup>. The present study of bioactive secondary metabolites revealed that *Aspergillus terreus* as a source for the production of effective metabolites. These metabolites can be further exploited for the biotechnological applications in medicine and agriculture.

*Antibacterial and antifungal activity*

Clinical pathogens selected for antibacterial activity namely, *Streptococcus pneumonia*, *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis*, *Staphylococcus epidermidis*, maximum zone formation against *Streptococcus pneumonia* ( $6.32 \pm 0.013$ ) mm, (table 2). In agar well diffusion method the selected medicinal plants were effective against *Aspergillus terreus*, Table 3. *Gramineae poaceae* was very highly active ( $7.47 \pm 0.14$ ) mm against *Aspergillus terreus*. *Aspergillus terreus* was found to be sensitive to all test medicinal plants and mostly comparable to the standard reference antifungal drug Amphotericin B and fluconazole to some extent. Previous studies Tayung et al., (2007)<sup>34</sup> showed that selection of different potential areas were important activity for isolation of different types of potent antibiotic producing endophytic fungi. *A. terreus* bioactivities recorded as 14.9 and 26.5 lg/ml against *V. ordalli* and *V. angularuim* respectively<sup>30</sup>. Because of the emergencies of multi-drug resistant pathogens, there are basic challenges for effective treatment for infectious disease. Thus, due to the burden for high frequency for multidrug resistant pathogens in the world, there has been increasing interest for searching effective antibiotics from soil actinomycetes in diversified ecological niches<sup>30</sup>. Kiran et al., (2007)<sup>35</sup> showed the marine fungus *Aspergillus ustus* MSF3 showed antimicrobial activity in fermentation medium at temperature of 20°C.

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