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Full Length Research Paper

Analysis of bioactive chemical compounds of *Aspergillus niger* by using gas chromatography-mass spectrometry and fourier-transform infrared spectroscopy

Imad Hadi Hameed*, Lena Fadhil Hamza and Sabreen A. Kamal

Department of Molecular Biology, Babylon University, Hilla City, Iraq.

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Bioactives are chemical compounds often referred to as secondary metabolites. Thirty five bioactive compounds were identified in the methanolic extract of *Aspergillus niger*. The identification of bioactive chemical compounds is based on the peak area, retention time molecular weight and molecular formula. Gas chromatography-mass spectrometry (GC-MS) analysis of *Aspergillus niger* revealed the existence of the 6-Acetyl-ß-d-mannose, 4-[Dichloromethyl]-2-[[2-[1-methyl-2-pyrrolidinyl]ethyl]amino-6-trichloro, 2-Furan-carboxaldehyde,5-methyl, 2,2,2-Trifluoro-N-[2-(1-hydroxy-2,2,6,6-tetramethyl-piperidin-4-yl), 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), Tetraacetyl-d-xylonic nitrile, Eicosanoic acid, phenylmethyl ester, Dodecanoic acid, 3-hydroxy, Desulphosinigrin, Glycyl-dl-serine, 2,5-Dimethyl-4-hydroxy-3(2H)-furanone, 2,5-Furandicarboxaldehyde, 2H-Oxecin-2-one,3,4,7,8,9,10- hexahydro-4-hydroxy-10-methyl, 6-Acetyl-ß-d-mannose, DL-Leucine, N-glycyl, 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl, l-Gala-l-ido-octonic lactone, 2H-Pyran,tetrahydro-2-(12-pentadecynyloxy), 5-Hydroxymethylfurfural, Strychane,1-acetyl-20α-hydroxy-16-methylene, α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3)ß-D-fru, Boroxin, tris(2,3-dimethylbut-2-yl), 16-Nitrobicyclo[10.4.0]hexadecane-1-ol-13-one, 3-[3-Bromophenyl]-7-chloro-3,4-dihydro-10-hydroxy-1,9(2H,10H)-a, Uric acid, 1,2,4-Trioxolane-2-octanoic acid ,5-octyl-,methyl ester, Tetraacetyl-d-xylonic nitrile, 1,2-Cyclopentanedicarboxylic acid, 4-(1,1-dimethylethyl)-,dimethyl, 2-Bromotetradecanoic acid, i-Propyl 11,12-methylene-octadecanoate, 1H-2,8a-Methanocyclopenta[a]cyclopropa [e]cyclodecan-11-one, and Octadecanoic acid. The FTIR analysis of *A. niger* proved the presence of aromatic rings, alkenes, aliphatic fluoro compounds, tetiary amine, C-N stretch, aromatic nitro compounds, ammonium ions and organic nitrate which shows major peaks at 696.30, 744.52, 821.68, 844.82, 900.76, 931.62, 1026.13, 1145.72, 1207.44, 1234.44, 1261.45, 1315.45, 1359.82, 1377.17, 1413.82, 1452.40, 1631.78, 1741.72, 2924.09, 3118.90, 3217.27 and 3271.27. *Datura stramonium* was very active against *A. niger*. Methanolic extract of bioactive compounds of *A. niger* were assayed for *in vitro* antibacterial activity against *Pseudomonas aerogenosa, Escherichia coli, Proteus mirabilis, Staphylococcus aureus* and *Klebsiella pneumonia* by using the diffusion method in agar. The zones of inhibition were compared with different standard antibiotics. The diameters of inhibition zones ranged from 0.46±0.1 to 6.52±0.61 mm for all treatments.

Key words: *Aspergillus niger*, bioactive compounds, gas chromatography-mass spectrometry, fourier-transform infrared spectroscopy.

INTRODUCTION

Aspergillus spp are ubiquitous opportunistic moulds that cause both allergic and invasive syndromes. The genus comprises approximately 180 species, of which 33 have been associated with human disease (Segal et al., 1998; Perfect et al., 2001). *Aspergillus niger* is the third most common species associated with invasive pulmonary aspergillosis (Bellini et al., 2003; Anupama et al., 2007). *A. niger* has a great economical and biotechnological interest and is extensively used for production of extracellular enzymes and organic acids such as citric acid (Baker, 2006; Perrone et al., 2007; Mogensen et al., 2010). It also produces fumonisin B2 (FB2) along with OTA. 9, 19, 27. Fumonisins are suspected to cause human and animal toxicoses, and are regarded as carcinogenic (Susca et al., 2010; Chacko et al., 2012; Gebreselema et al., 2013). A culture yielding *Aspergillus* spp, in addition to enabling a diagnosis of invasive aspergillosis, may further define therapeutic options via susceptibility testing or the isolation of a species possessing inherent antifungal resistance; examples of the latter include *Aspergillus terreus* and *Aspergillus nidulans*, which are both resistant to amphotericin B (Walsh, 2004). The main disadvantage of culture is that it is relatively slow (the process takes days), is relatively insensitive, and requires specialized expertise for species determination.

In common with other pathogenic fungi, the ability to grow at 37°C distinguishes *Aspergillus* spp from other nonpathogenic environmental moulds. *Aspergillus* spp can be recovered on most routine solid and liquid microbiological media (example, blood agar, chocolate agar, brain heart infusion broth). A fungal-specific medium example, sabouraud dextrose agar should be included at the time of initial specimen set-up in clinical scenarios in which *Aspergillus* spp (or other moulds) are considered possible pathogens, because of superior yield (Horvath and Dummer, 1995). The addition of antibiotics example, chloramphenicol and gentamicin to the medium is required for the recovery of *Aspergillus* spp from specimens obtained from nonsterile sites, since they prevent bacterial overgrowth. Cycloheximide, a eukaryotic protein synthesis inhibitor, is frequently added to fungal media to inhibit the overgrowth of cultures by non-pathogenic environmental moulds; however, on occasion, cycloheximide may inhibit the growth of *Aspergillus* spp. The aim of this study were analysis of the secondary metabolites and the evaluation of antibacterial and antifungal activity .

MATERIALS AND METHODS

Collection and growth condition

A. niger was isolated from dried fruit and the pure colonies were selected, isolated and maintained in potato dextrose agar slants (Usha and Masilamani, 2013). After the species were identified by the identification key, 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.

Production, extraction and determination of metabolites

The metabolites were determined and extracted for gas chromatography (GC) analysis using the method of Siddiquee et al. (2012) with some modifications. 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 45ºC. The residue was dissolved in 1 ml methanol, filtered through a 0.2 µm syringe filter, and stored at 4ºC for 24 h before being used for gas chromatography–mass spectrometry (GC-MS) (Imad et al., 2014a). 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.

GC-MS analysis

Bioactive compound were examined for the chemical composition using GC-MS (Agilent 789N) equipped with a DB-5MS column (30 m×0.25 mm i.d., 0.25 um film thickness, J&W Scientific, Folsom, CA). The oven temperature was programmed as for the previous analysis (Imad et al., 2015a; Muhanned et al., 2015). 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 to 450 amu. The constituents were identified after being compared with available data in the GC-MS library in the literatures (Imad et al., 2015b; Mohammed et al., 2013).

Fourier transform infrared spectrophotometer (FTIR)

The powdered sample of the *A. niger* specimen was treated for fourier transform infrared spectroscopy (Shimadzu, IR Affinity 1, Japan). The sample was run at infrared region between 400 nm and 4000 nm.

Determination of antibacterial activity of crude fraction of *A. niger* compounds

The test pathogens (*E. coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Staphylococcus aureus)* were swabbed in Muller Hinton agar plates. 90µl of fungal extracts was loaded on the bored wells. The wells were bored in 0.5cm in diameter. The plates were incubated at 37°C for 24 h and examined. After the incubation the diameter of inhibition zones around the discs was measured.

*Corresponding author. E-mail: imad_dna@yahoo.com.

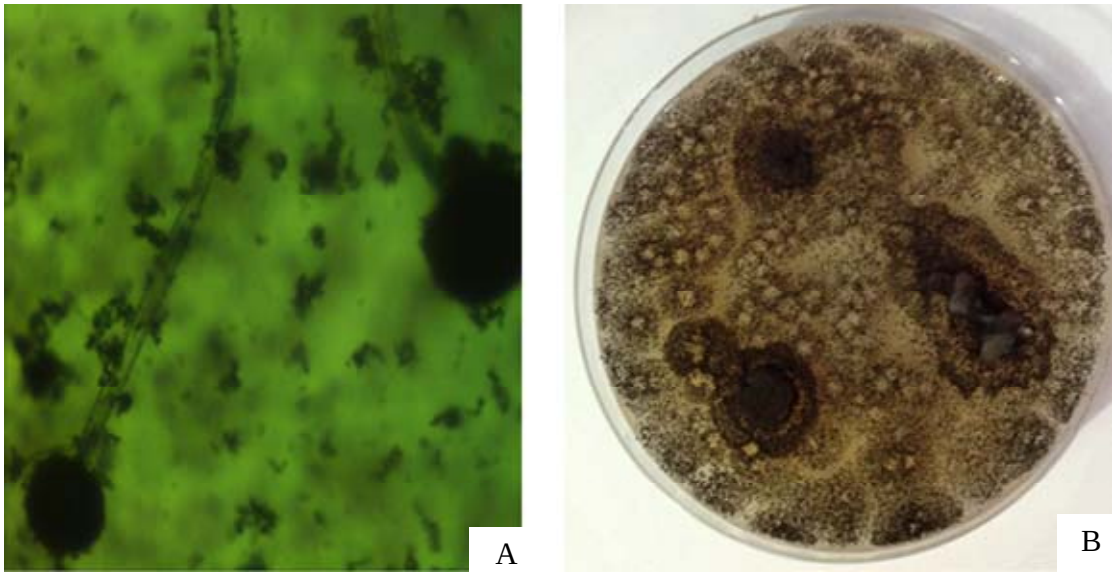


Figure 1. Morphological characterization of *Aspergillus niger*. (B) Microscopic observation (A) colony.

Determination of antifungal activity

A. niger 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 (Perez et al., 1990; Perez et al., 1999; Erdemogllu et al., 2003; Bagamboula et al., 2004). Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 25 µl of the samples solutions (*Nerium olender, Ricinus communis, Datura stramonium*, *Linum usitatissimum*, *Anastatica hierochuntica* and *Gramineae poaceae*) were delivered into the wells. The plates were incubated for 48 h at room temperature (Huda et al., 2015a; Ameera et al., 2015; Imad et al., 2015c). 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 (Anesini and Perez, 1993; Rukayadi et al., 2006; Huda et al., 2015b). The tests were carried out in triplicate. The antifungal activity was evaluated by measuring the inhibition-zone diameter observed after 48 h of incubation.

Statistical analysis

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 statistical package for the social sciences (SPSS) software) Version 9.1 (Imad et al., 2014b).

RESULTS AND DISCUSSION

Isolation of fungi from dried fruit

The fungi were isolated from dried fruit by serial dilution method. Based on morphological, characteristics of fungi was isolated in selective media of potato dextrose agar media. Morphological, Microscopical and microscopical characteristics of fungal strains were determined using specific media light and compound microscope Figure 1.

Production and Identification of secondary metabolites from the methanolic crude extract of *A. niger* by gas chromatography and mass spectrometry and fourier-transform infrared spectroscopy

The 400 ml of fermentation broth (PDA broth) which contain 200 µl 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 *A. niger*, as shown in Table 1. The GC-MS chromatogram of the seventeen peaks of the compounds detected was shown in Figure 2. Chromatogram GC-MS analysis of the methanol extract of *A. niger* showed the presence of twenty major peaks and the components corresponding to the peaks were determined as follows. The First set up peak were determined to be 6-Acetyl-ß-d-mannose (Figure 3). The second peak indicated to be 4-[Dichloromethyl]-2-[[2-[1-methyl-2-pyrrolidinyl]ethylamino-6-trichloro (Figure 4). The next peaks considered to be 2-Furan-carboxaldehyde, 5-methyl, 2,2,2-Trifluoro-N-[2-(1-hydroxy-2,2,6,6-tetramethyl-piperidin-4-yl), 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, HEPES, Tetraacetyl-d-xylonic nitrile, eicosanoic acid, phenylmethyl ester, dodecanoic acid , 3-hydroxy, Desulphosinigrin,

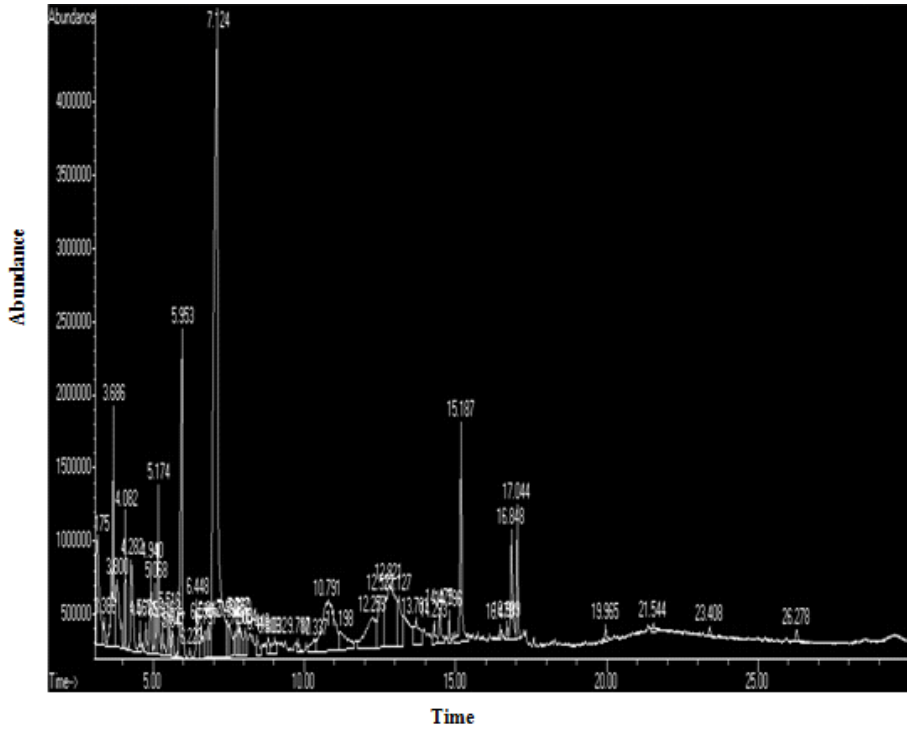


Figure 2. GC-MS chromatogram of methanolic extract of *A. niger.*

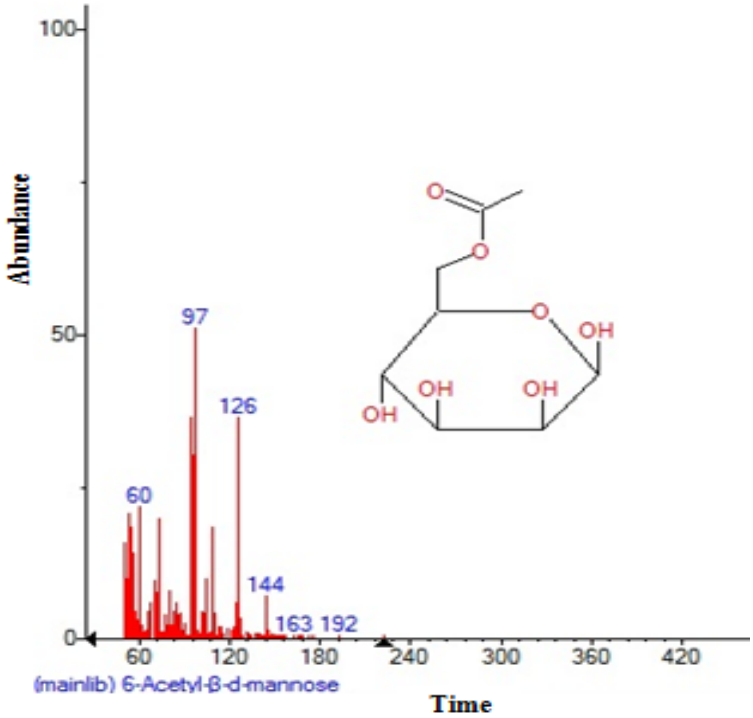


Figure 3. Mass spectrum of 6-Acetyl-ß-d-mannose with Retention Time (RT)= 3.201.

Glycyl-dl-serine, 2,5-Dimethyl-4 -hydroxy-3(2H)-furanone, 2,5-Furandicarboxaldehyde, 2H-oxecine-2-one, 3,4,7,8,9

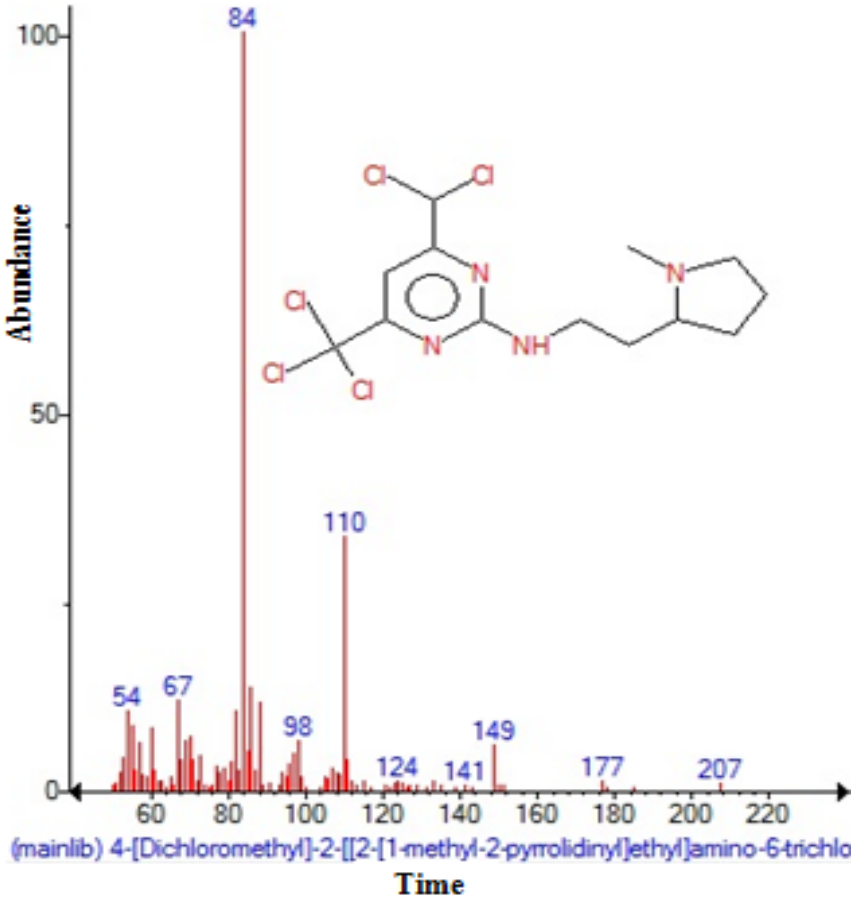


Figure 4. Mass spectrum of 4-[Dichloromethyl]-2-[[2-[1-methyl-2-pyrrolidinyl]ethyl]amino-6-trichloro with Retention Time (RT)= 3.613.

10-hexahydro-4-hydroxy-10-methyl, 6-Acetyl-ß-d-mannose, DL-Leucine, N-glycyl, 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl, l-Gala-l-ido-octonic lactone, 2H-Pyran,tetrahydro-2-(12-pentadecynyloxy), 5-Hydroxymethylfurfural, Strychane,1-acetyl-20α-hydroxy-16-methylene, α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3)ß-D-fru, Boroxin, tris (2,3-dimethylbut-2-yl), 16-Nitrobicyclo[10.4.0]hexadecane-1-ol-13-one, 3-[3-Bromophenyl]-7-chloro-3,4-dihydro-10-hydroxy-1,9(2H,10H)-a, Uric acid, 1,2,4-Trioxolane-2-octanoic acid ,5-octyl-,methyl ester, Tetraacetyl-d-xylonic nitrile, 1,2-Cyclopentanedicarboxylic acid ,4-(1,1-dimethylethyl)-,dimethyl, 2-Bromotetradecanoic acid, i-Propyl 11,12-methylene-octadecanoate, 1H-2,8a-Metha-nocyclopenta[a]cyclopropa[e]cyclodecan-11-one and Octadecanoic acid (Figures 5 to 34). Many compounds are identified in the present study. Some of them are biological compounds with antimicrobial activities.

Fourier-transform infrared analysis of dry methanolic extract of *A. niger* proved the presence of aromatic rings, alkenes, aliphatic fluoro compounds, alcohols, ethers, carboxlic acids, esters, nitro compounds, aldehydes, ketones, alkanes, hydrogen bonded alcohols and phenols compounds shows major peaks at 894.97, 927.76, 1024.20, 1236.37, 1317.38, 1608.63, 2306.86, 2850.79, 2922.16, 3184.48, 3277.06 and 3292.49, respectively. (Table 2 and Figure 35).

Antibacterial activity

Four clinical pathogens selected for antibacterial activity namely, *k. pneumoniae, P. aeroginosa, E. coli, S. aeureus.* and maximum zone formation against *k. pneumonia* (Table 3 and Figure 36).

Antifungal activity

Each extract plant showed notable antifungal activities against *A. niger* (Figure 37). In agar well diffusion method the selected medicinal plants (*N. olender, R. communis, D. stramonium*, *L. usitatissimum*, *A. hierochuntica* and *G.*

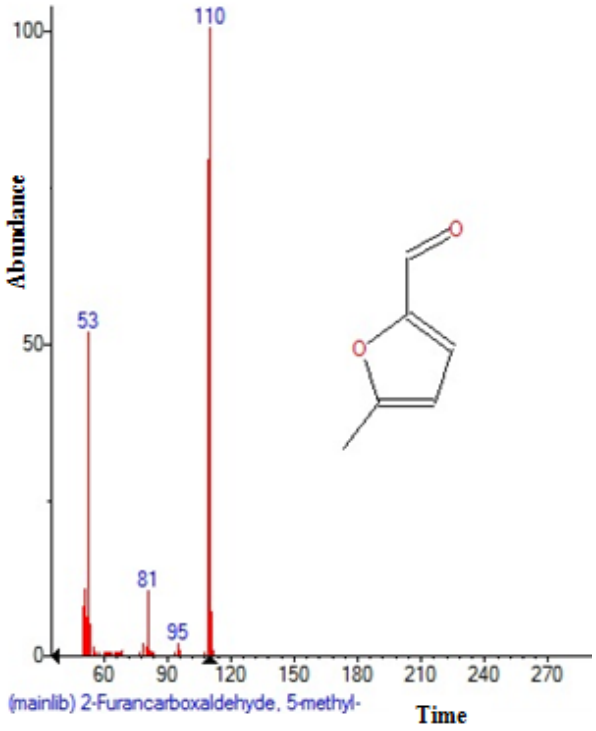


Figure 5. Mass spectrum of 2-Furancarboxaldehyde,5-methyl with Retention Time (RT)= 3.722.

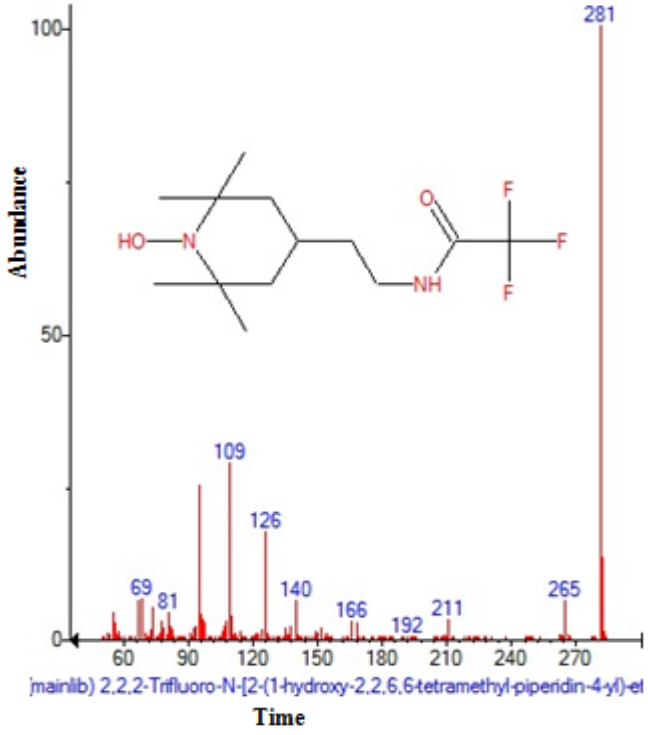


Figure 6. Mass spectrum of 2,2,2-Trifluoro-N-[2-(1-hydroxy-2,2,6,6-tetramethyl-piperidin-4-yl)-el with Retention Time (RT)= 3.779.

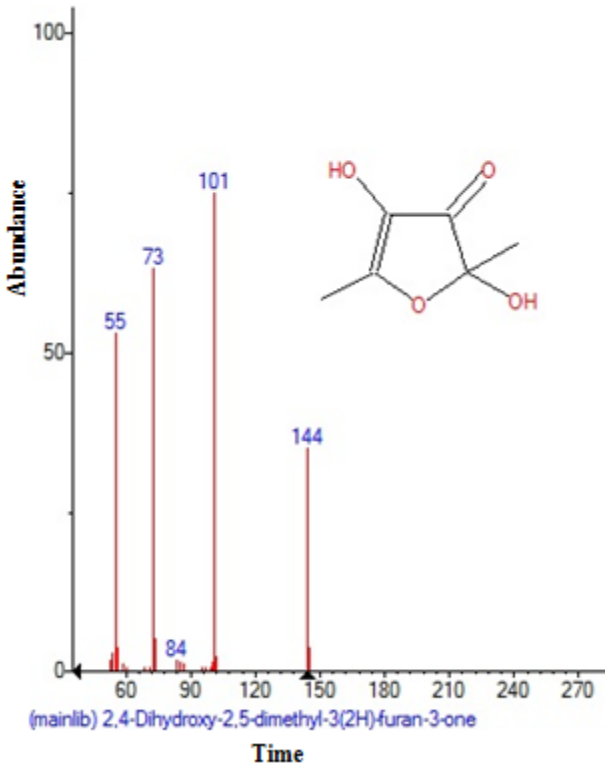


Figure 7. Mass spectrum of 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one with Retention Time (RT)= 4.076.

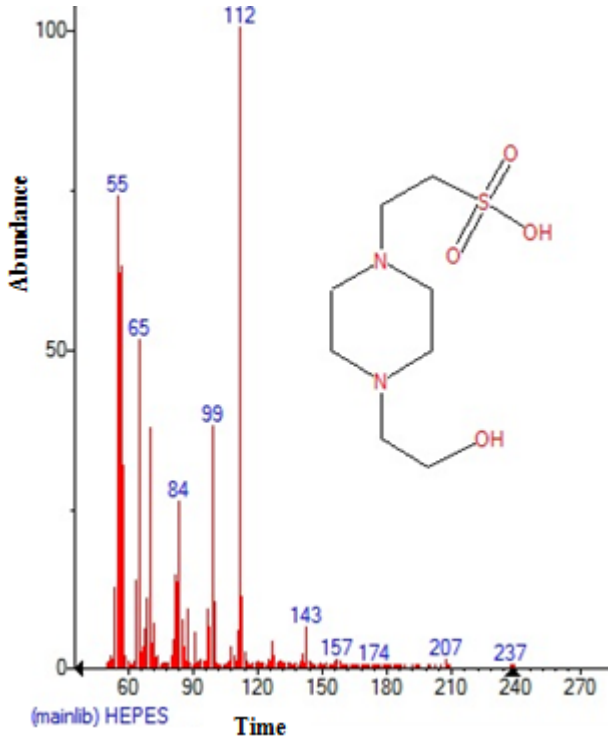


Figure 8. Mass spectrum of 2 HEPES with Retention Time (RT)= 4.271.

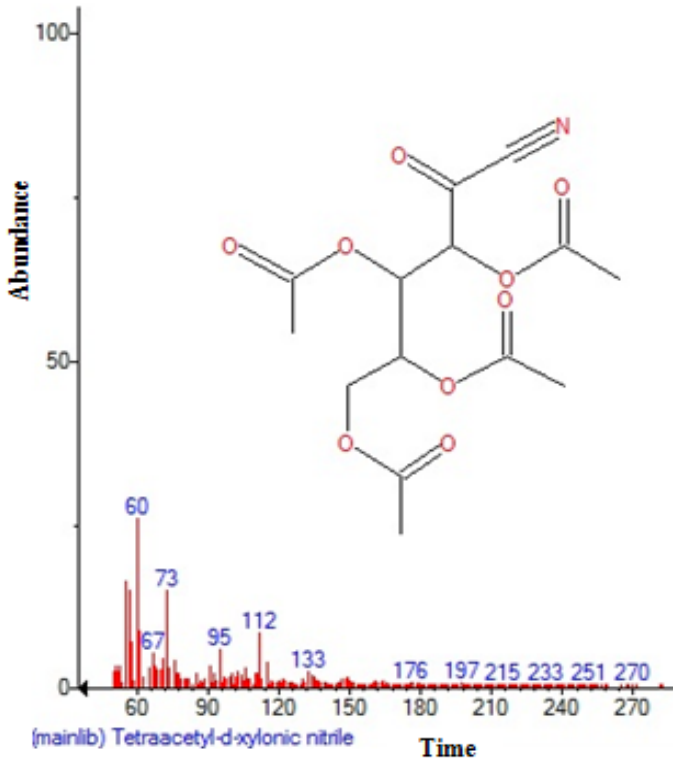


Figure 9. Mass spectrum of Tetraacetyl-d-xylonic nitrile with Retention Time (RT)= 4.465.

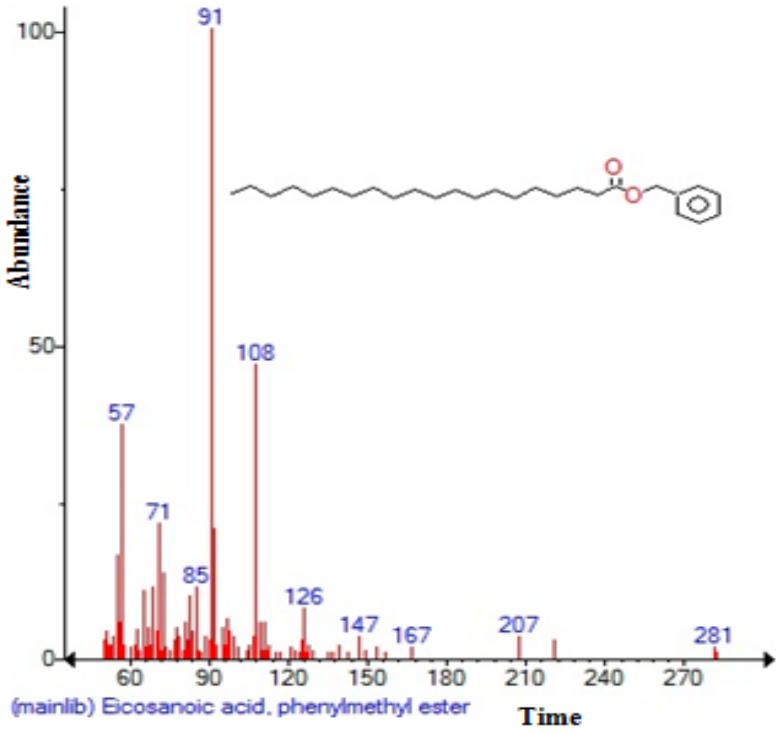


Figure 10. Mass spectrum of eicosanoic acid , phenylmethyl ester with retention time (RT)= 4.546.

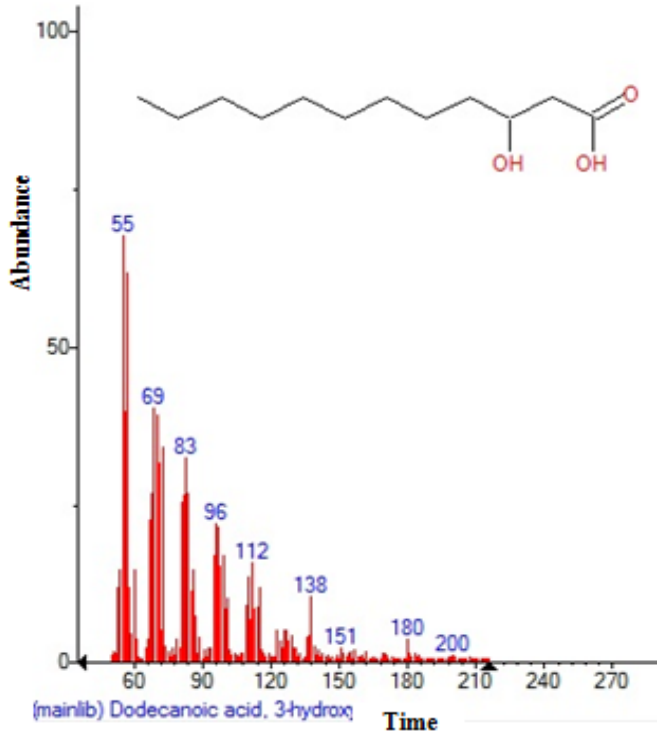


Figure 11. Mass spectrum of dodecanoic acid , 3-hydroxy with retention time (RT)= 4.574.

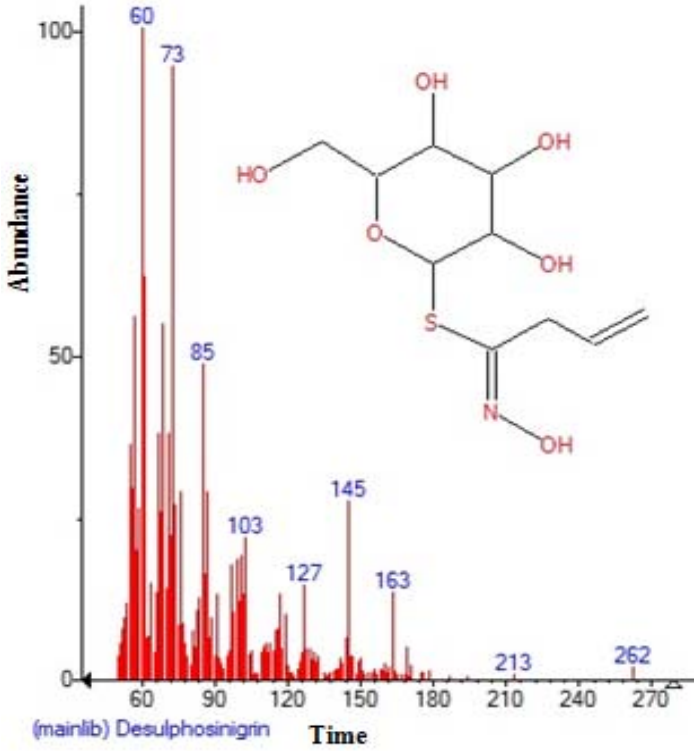


Figure 12. Mass spectrum of desulphosinigrin with retention time (RT)= 4.654.

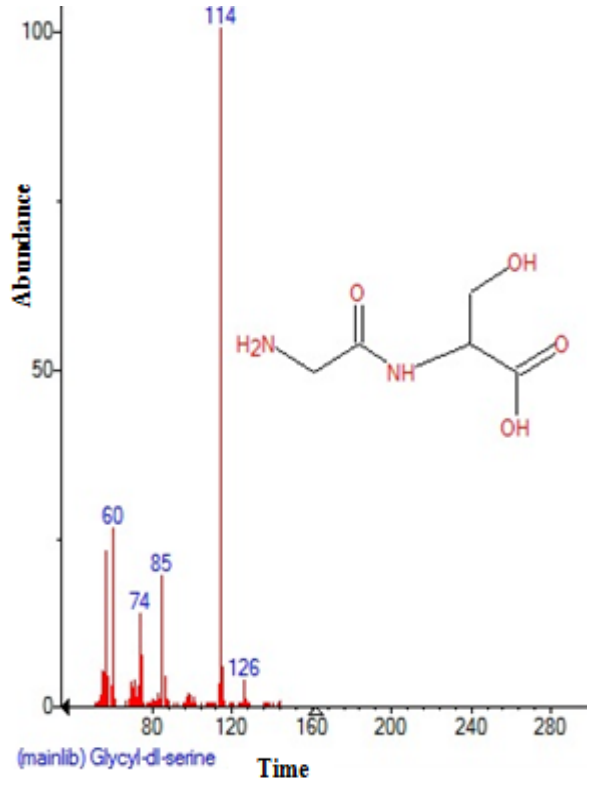


Figure 13. Mass spectrum of Glycyl-dl-serine with retention time (RT)= 4.763.

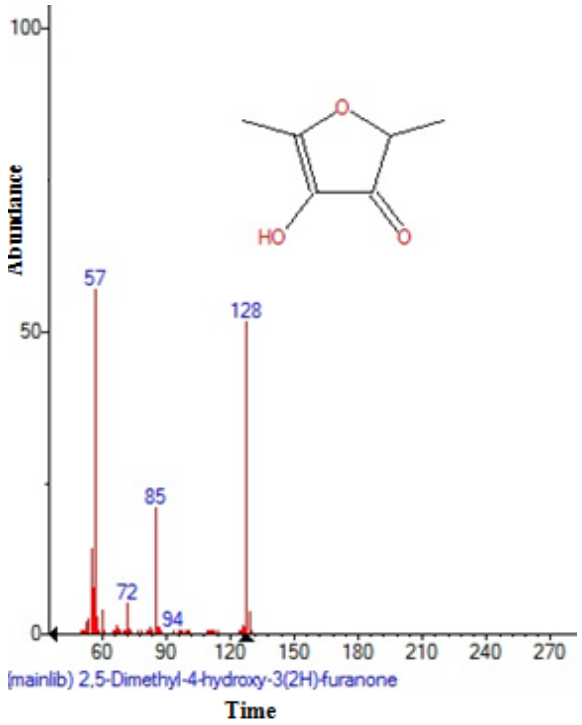


Figure 14. Mass spectrum of 2,5-Dimethyl-4-hydroxy-3(2H)-furanone with retention time (RT)= 4.929.

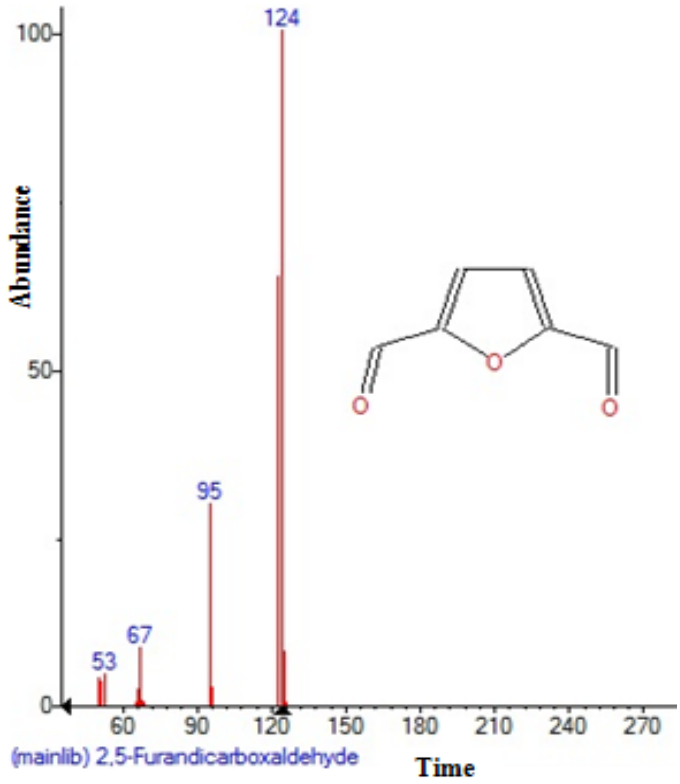


Figure 15. Mass spectrum of 2,5-Furandicarboxaldehyde with retention time (RT)= 5.066.

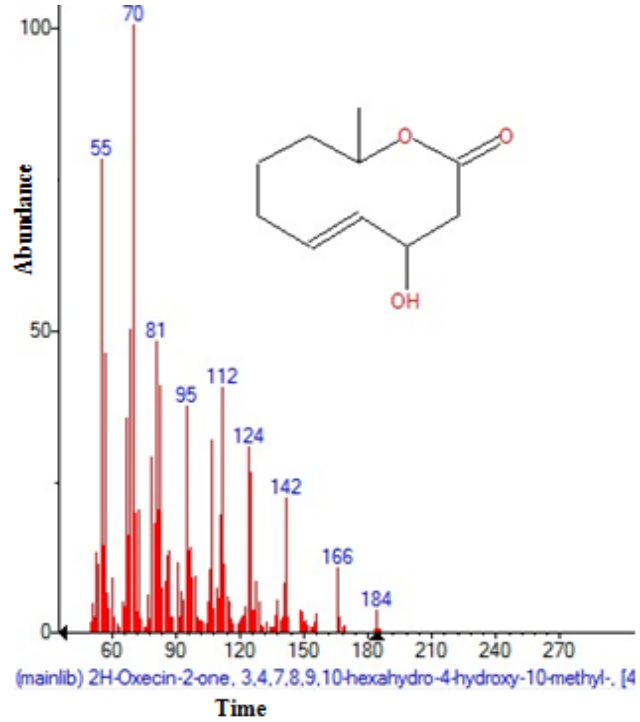


Figure 16. Mass spectrum of 2H-Oxecin-2-one,3,4,7,8,9,10-hexahydro-4-hydroxy-10-methyl-,[4 with retention time (RT)= 5.261.

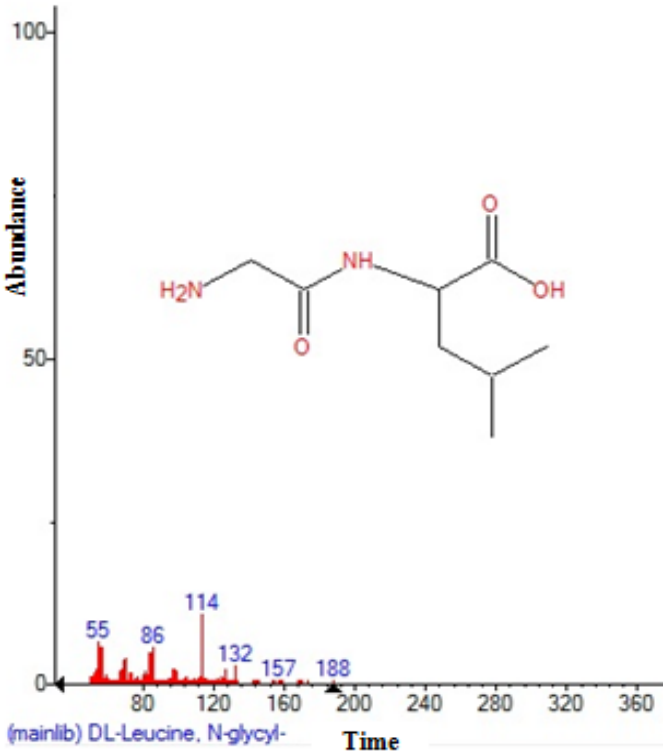


Figure 17. Mass spectrum of DL-Leucine , N-glycyl with retention time (RT)= 5.616.

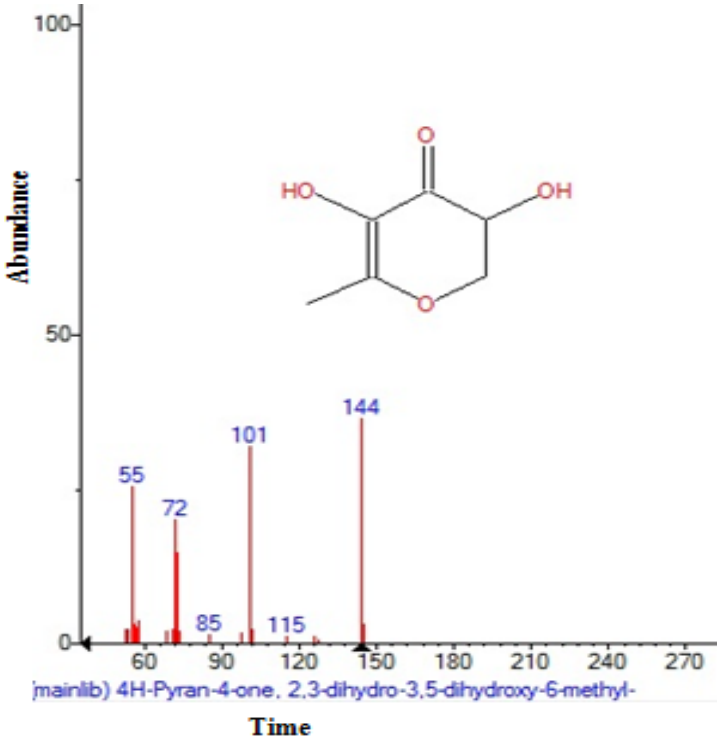


Figure 18. Mass spectrum of 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl with retention time (RT)= 5.942.

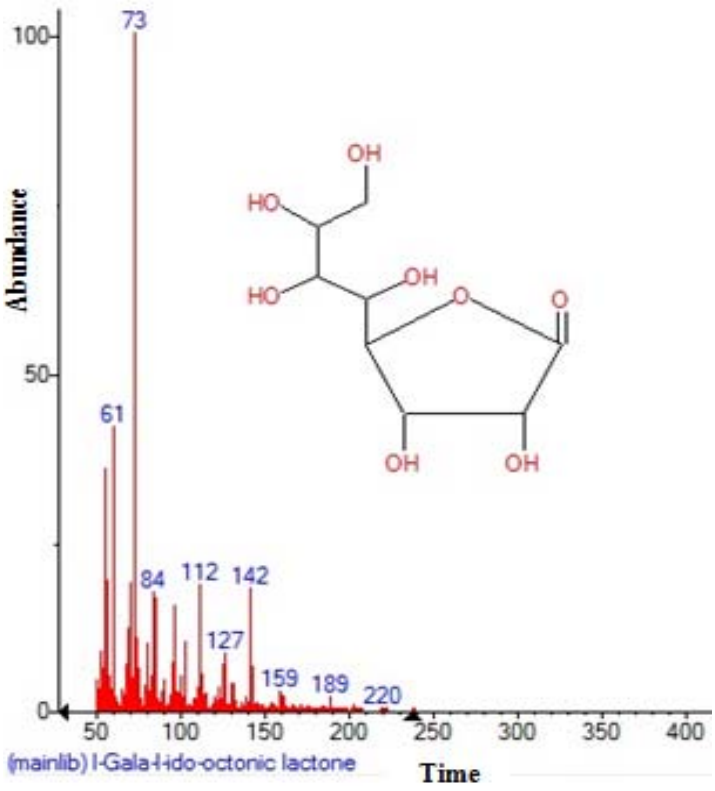


Figure 19. Mass spectrum of l-Gala-l-ido-octonic lactone with retention time (RT)= 6.577.

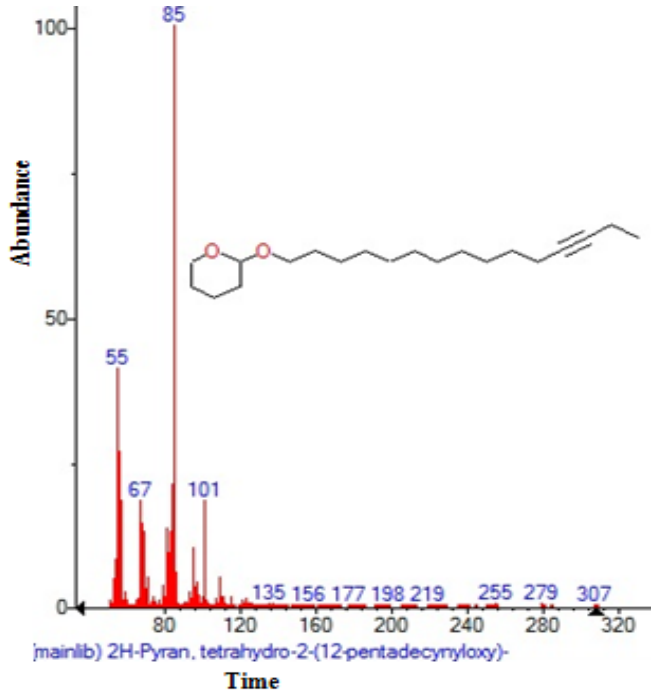


Figure 20. Mass spectrum of 2H-Pyran,tetrahydro-2-(12-pentadecynyloxy) with retention time (RT)= 6.737.

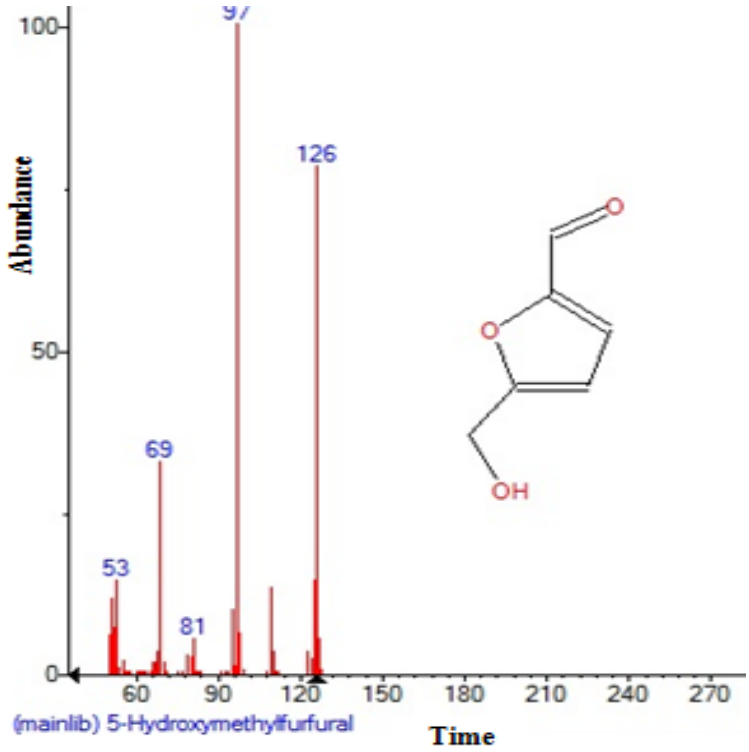


Figure 21. Mass spectrum of 5-Hydroxymethylfurfural with retention time (RT)= 7.120.

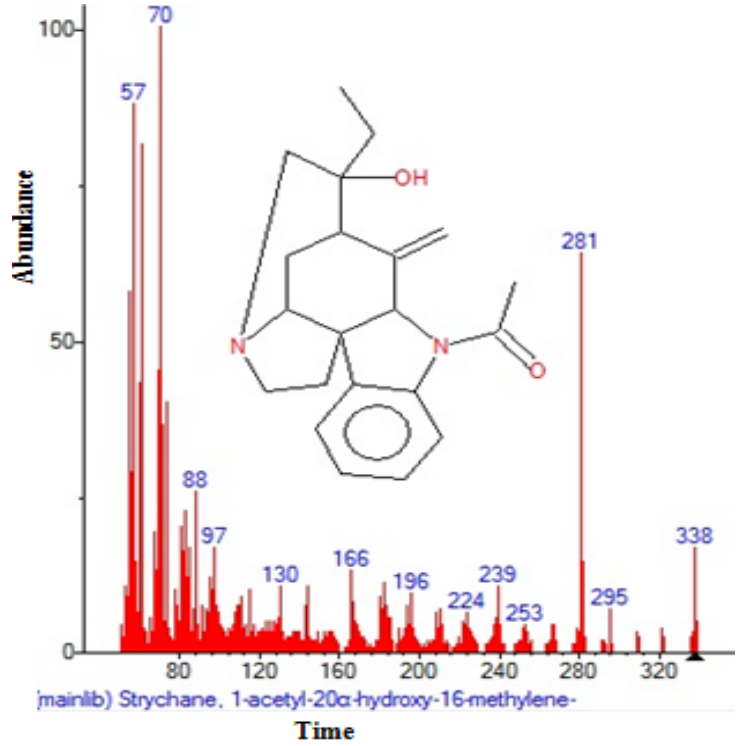


Figure 22. Mass spectrum of Strychane,1-acetyl-20α-hydroxy-16-methylene with retention time (RT)= 8.053.

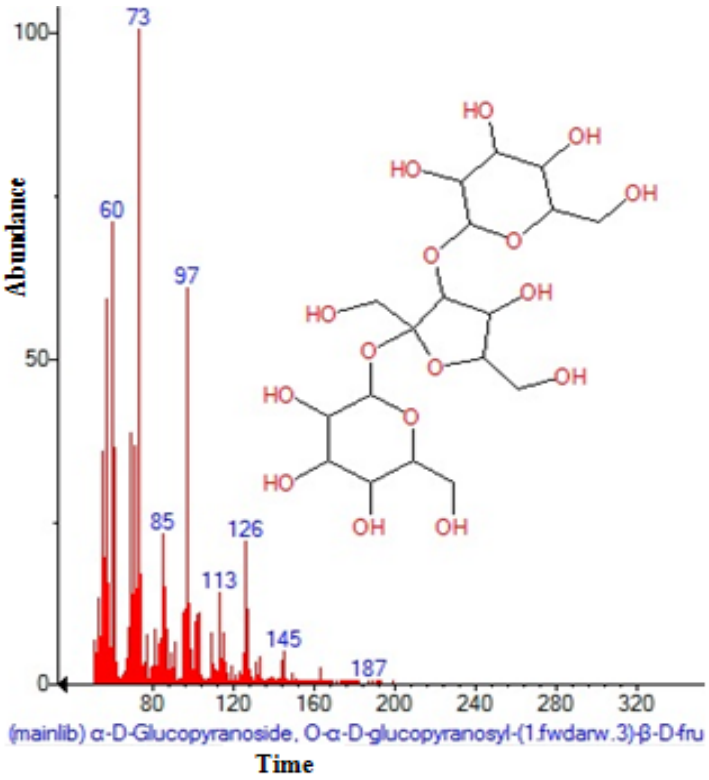


Figure 23. Mass spectrum of α-D-Glucopyranoside ,O-α-D-glucopyranosyl-(1.fwdarw.3)ß-D-fru with retention time (RT)= 7.836.

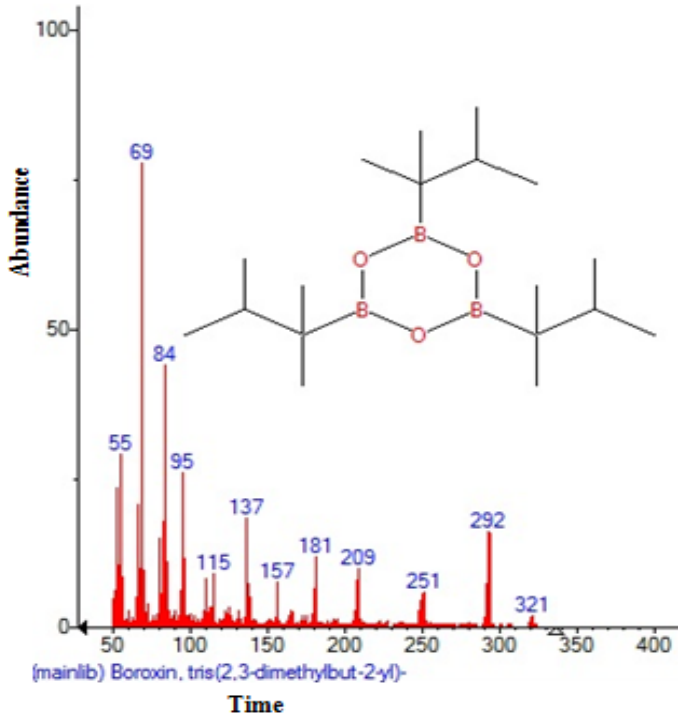


Figure 24. Mass spectrum of Boroxin , tris(2,3-dimethylbut-2-yl) with retention time (RT)= 8.442.

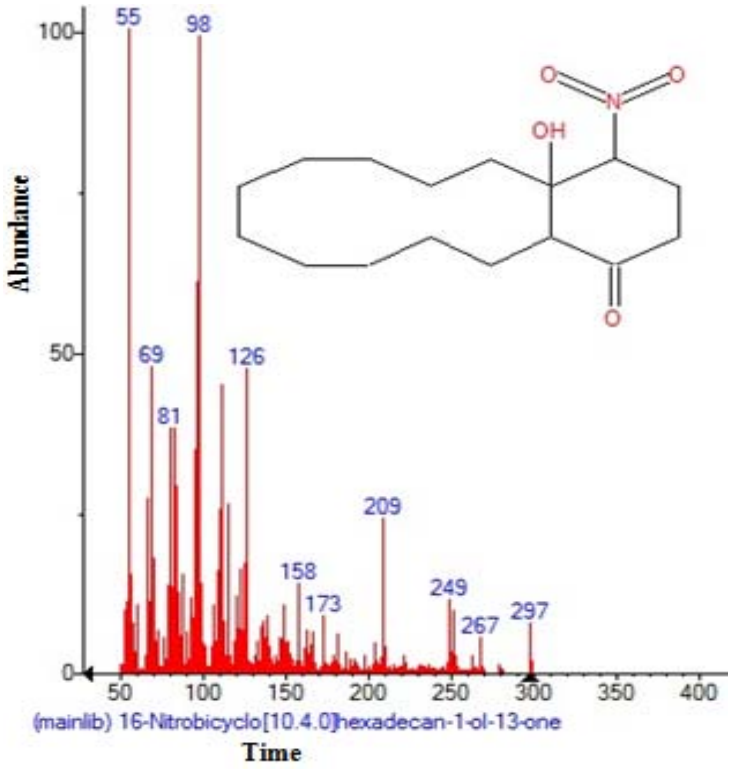


Figure 25. Mass spectrum of 16-Nitrobicyclo[10.4.0]hexadecane-1-ol-13-one with retention time (RT)= 8.797.

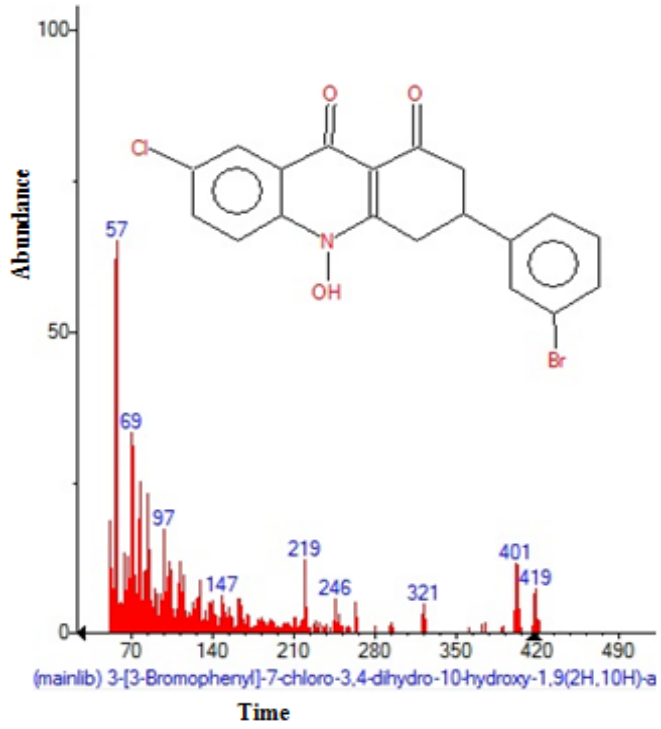


Figure 26. Mass spectrum of 3-[3-Bromophenyl]-7-chloro-3,4-dihydro-10-hydroxy-1,9(2H,10H)-a with retention time (RT)= 9.043.

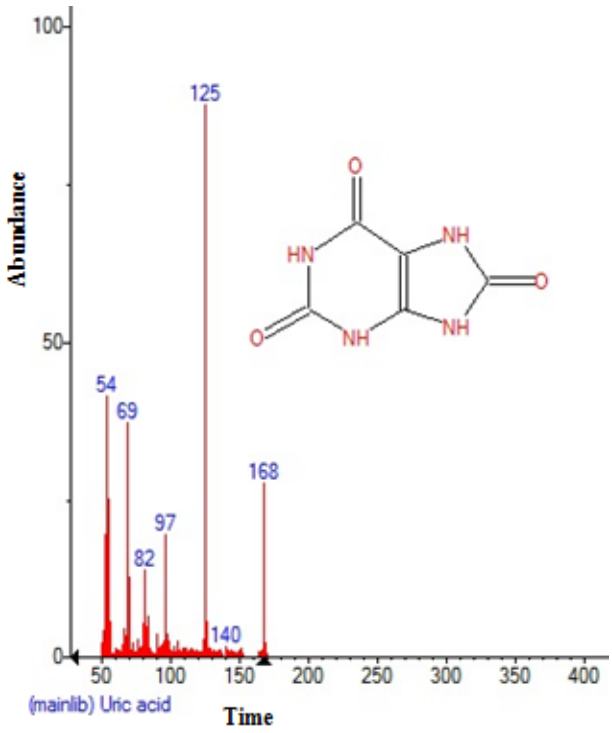


Figure 27. Mass spectrum of uric acid with retention time (RT)= 9.672.

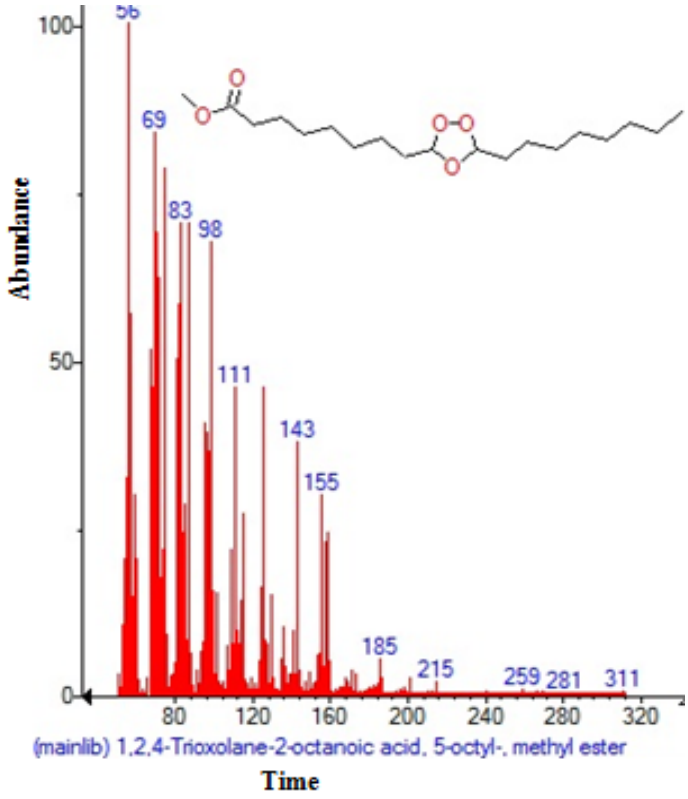


Figure 28. Mass spectrum of 1,2,4-Trioxolane-2-octanoic acid ,5-octyl-,methyl ester with retention time (RT)= 11.320.

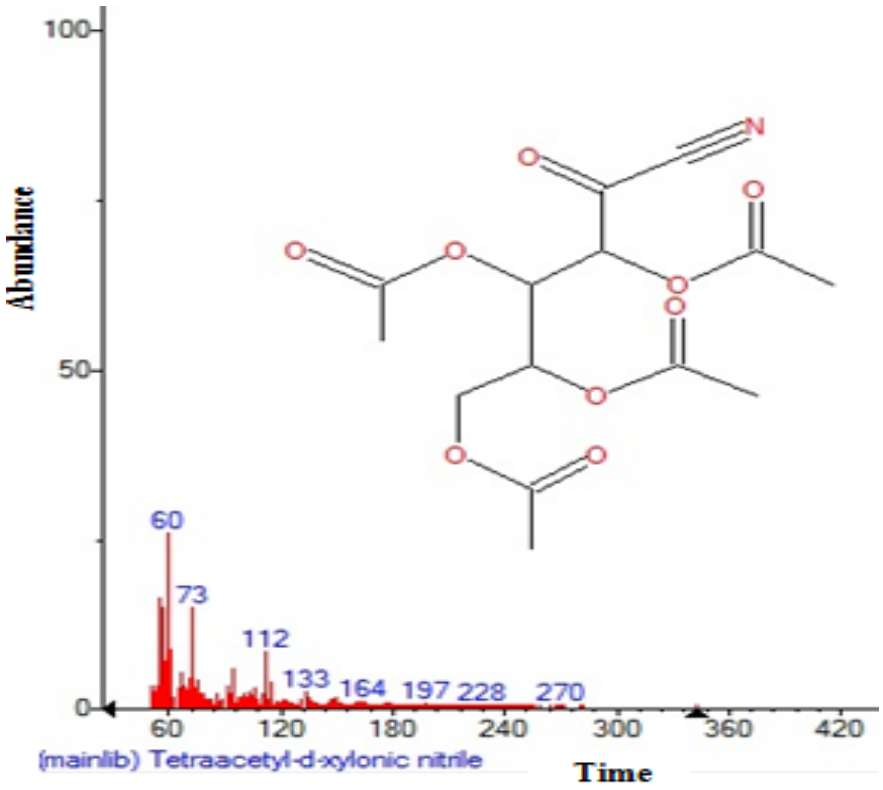


Figure 29. Mass spectrum of Tetraacetyl-d-xylonic nitrile with retention time (RT)= 12.276.

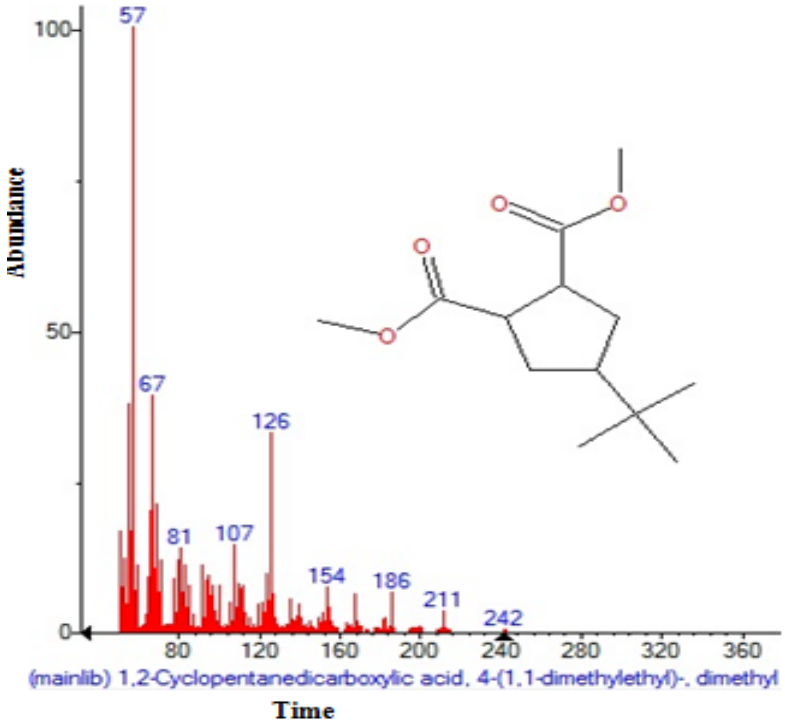


Figure 30. Mass spectrum of 1,2-Cyclopentanedicarboxylic acid ,4-(1,1-dimethylethyl)-,dimethyl with retention time (RT)= 13.975.

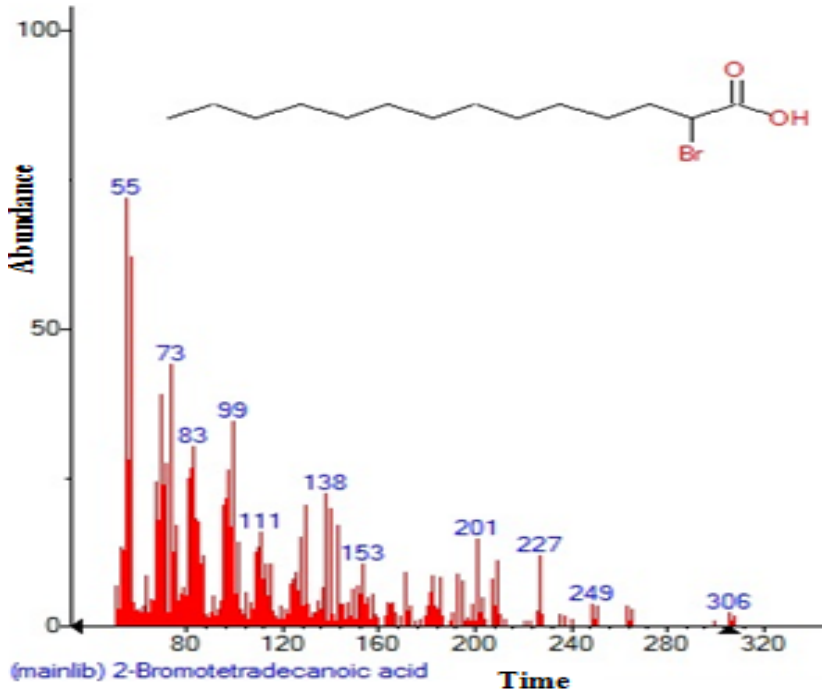


Figure 31. Mass spectrum of 2-Bromotetradecanoic acid with retention time (RT)= 14.771.

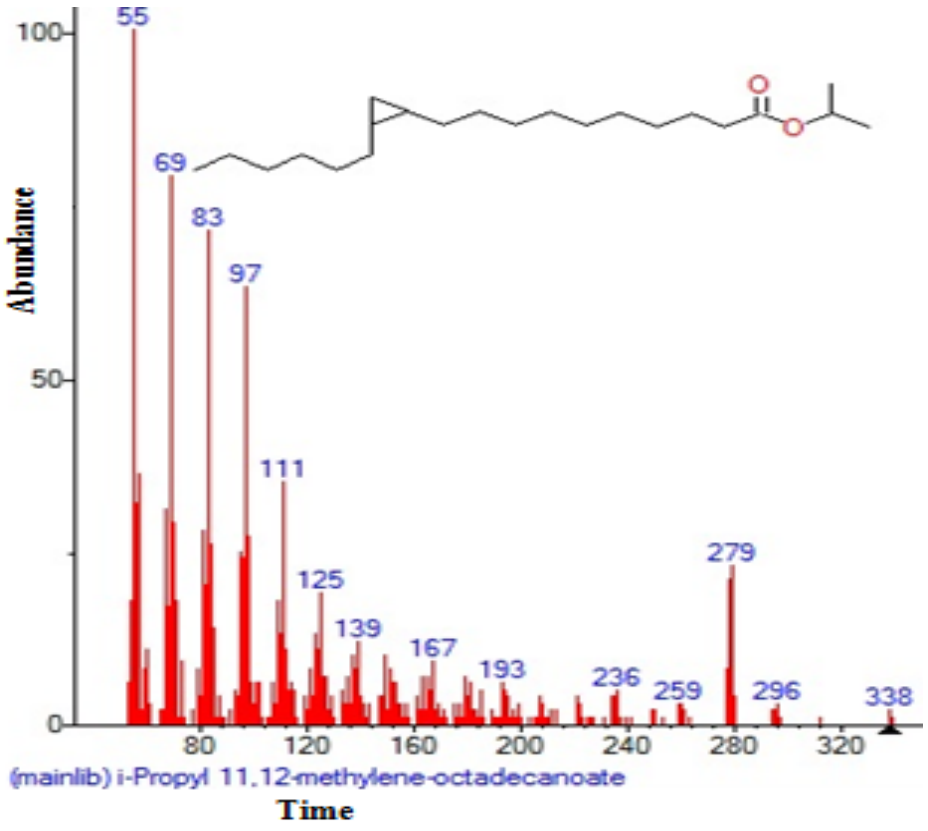


Figure 32. Mass spectrum of i-Propyl 11,12-methylene-octadecanoate with retention time (RT)= 15.022.

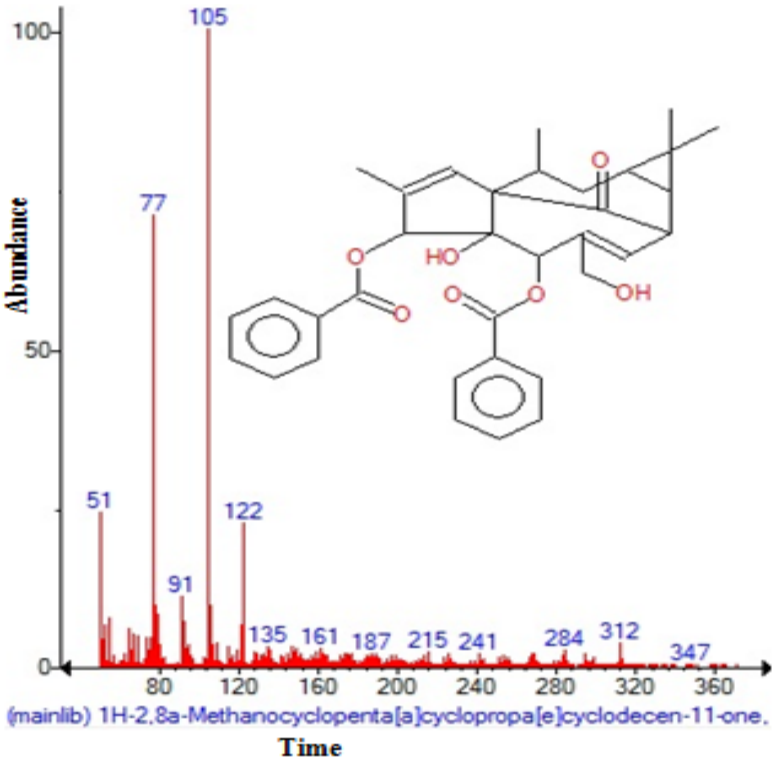


Figure 33. Mass spectrum of 1H-2,8a-Methanocyclopenta[a]cyclopropa[e]cyclodecan-11-one with retention time (RT)= 17.214.

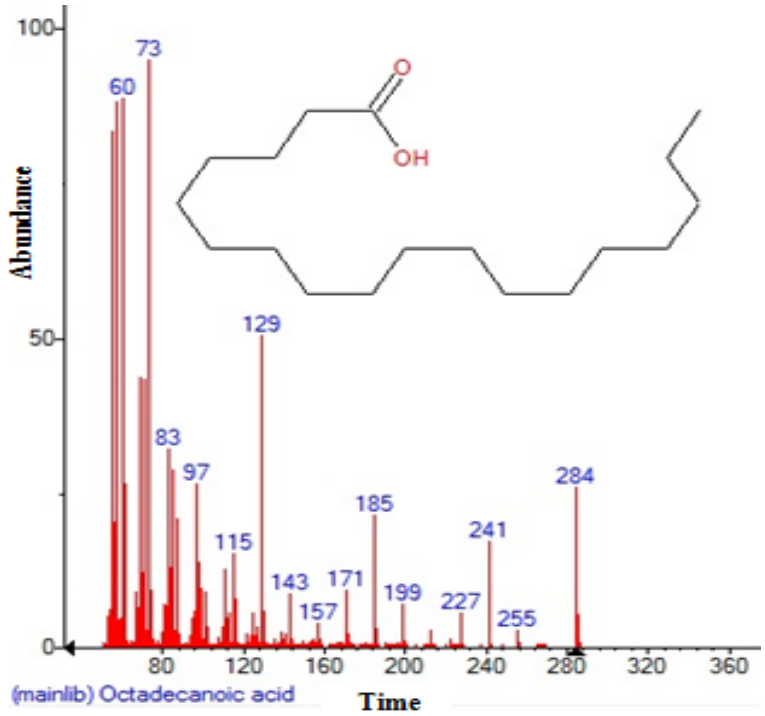


Figure 34. Mass spectrum of octadecanoic acid with retention time (RT) = 17.048.

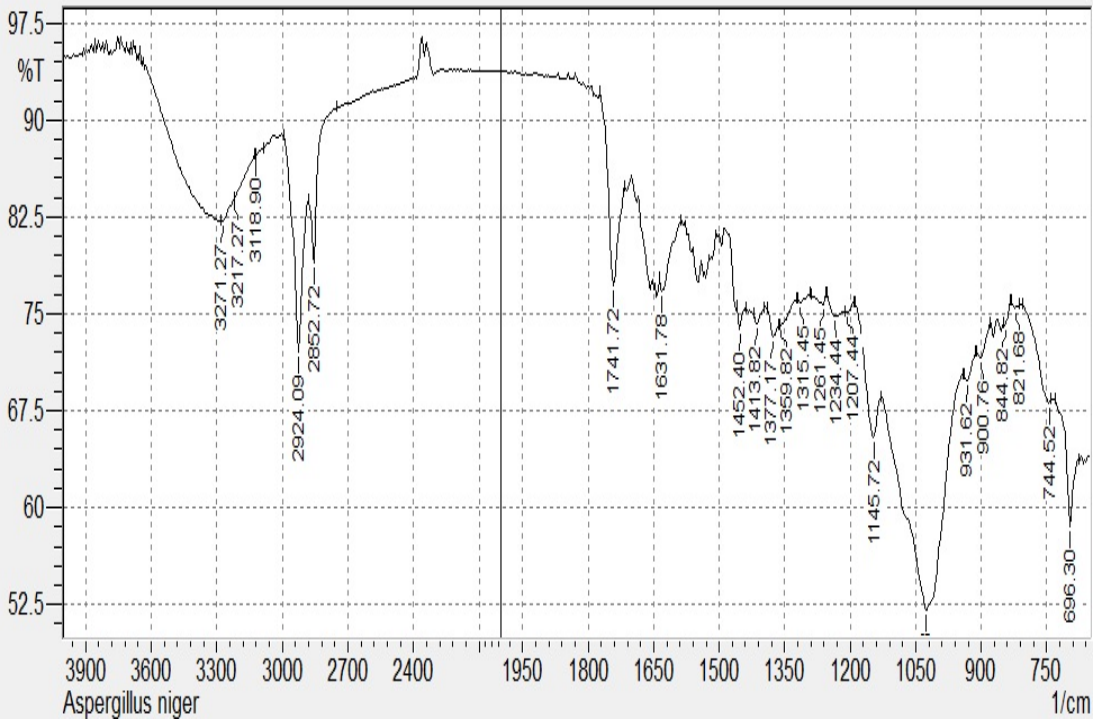


Figure 35. Fourier-transform infrared spectroscopy peak values of *A. niger*

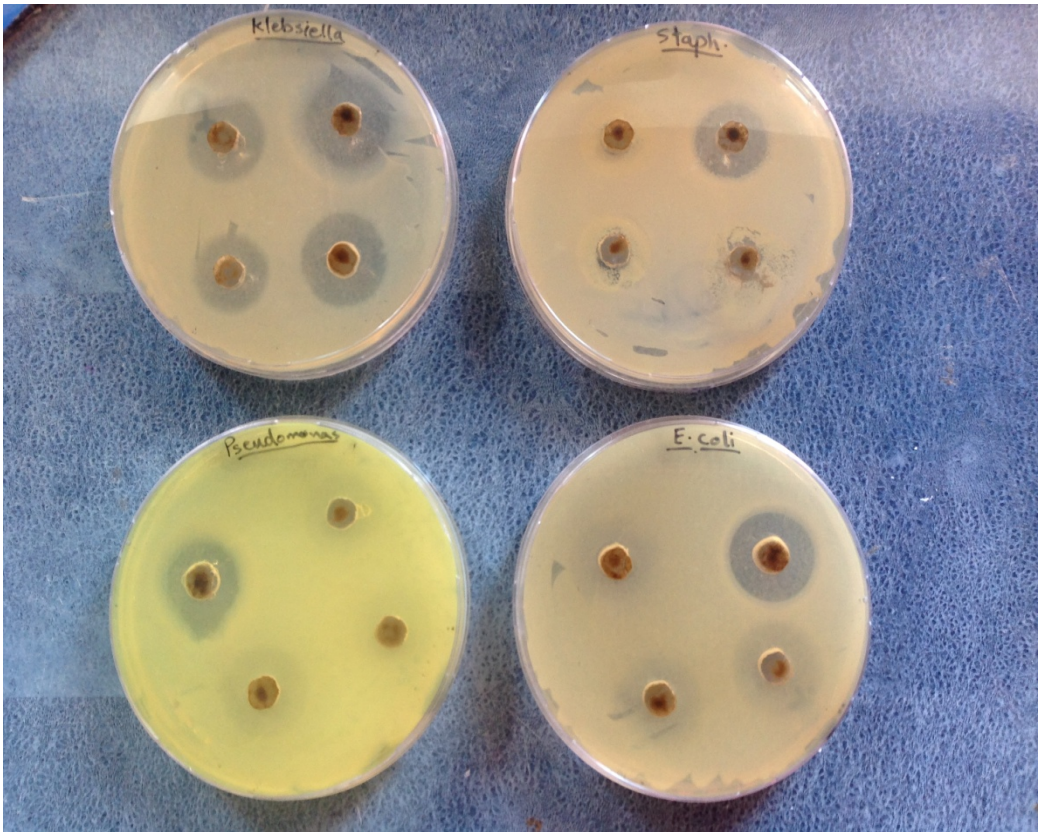
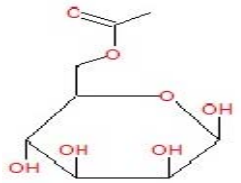
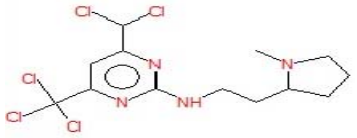
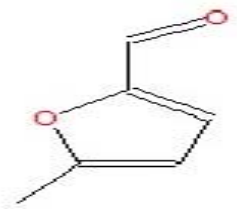
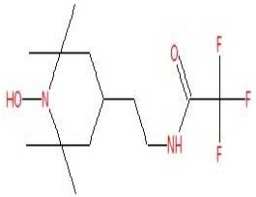


Figure 36. Antimicrobial activity of *A. niger.*

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

S/N	Phytochemical compound	RT (min)	Formula	Molecular weight	Exact mass	Chemical structure	MS Fragment- ions
1	6-Acetyl-ß-d-mannose	3.201	$C_8H_{14}O_7$	222	222.073953		60, 97, 126, 144, 163, 192
2	4-[Dichloromethyl]-2-[[2-[1-methyl-2-pyrrolidinyl]ethyl]amino-6-trichloro	3.613	$C_{13}H_{17}Cl_5N_4$	403	403.989586		54, 67, 84, 98, 110, 124, 141, 149, 177, 207
3	2-Furancarboxaldehyde,5-methyl	3.722	$C_6H_6O_2$	110	110.0367794		53, 81, 95, 110
4	2,2,2-Trifluoro-N-[2-(1-hydroxy-2,2,6,6-tetramethyl-piperidin-4-yl)	3.779	$C_{13}H_{23}F_3N_2O_2$	296	296.171164		69, 81, 109, 126, 140, 166, 192, 211, 265, 281

poaceae) were effective against *A. niger* (Table 4). *D. stramonium* was very highly active against *A. niger*. *A. niger* 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.

CONCLUSION

The results of this study showed that *A. niger*

Table 1. Contd.

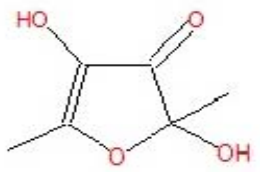
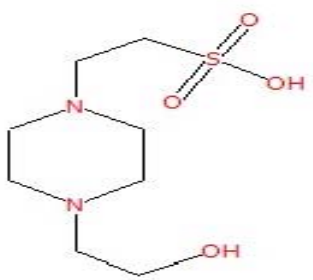
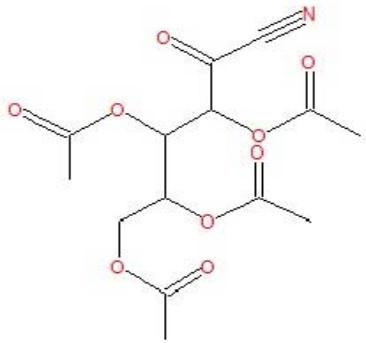
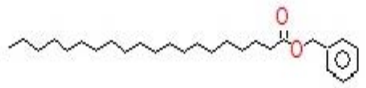
5	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	4.076	$C_6H_8O_4$	144	144.042258		55, 73, 84, 101,144
6	HEPES	4.271	$C_8H_{18}N_2O_4S$	238	238.098728		55, 65, 84, 99, 112, 143, 157, 174, 207, 237
7.	Tetraacetyl-d-xylonic nitrile	4.465	$C_{14}H_{17}NO_9$	343	343.090332		60, 67, 73, 95, 112, 133, 176, 197, 215, 233, 251, 270
8	Eicosanoic acid , phenylmethyl ester	4.546	$C_{27}H_{46}O_2$	402	402.349781		57, 71, 85, 91, 108, 126, 147, 167, 207, 281

Table 1. Contd.

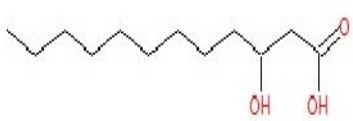
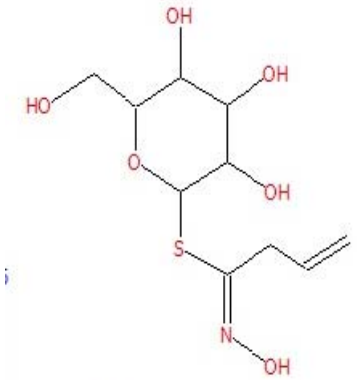
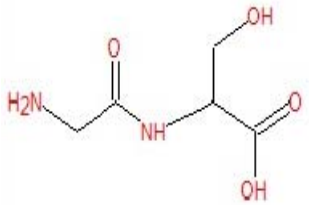
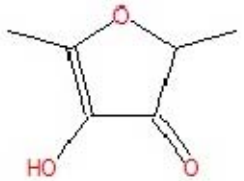
9	Dodecanoic acid , 3-hydroxy	4.574	$C_{12}H_{24}O_3$	216	216.125445		55, 69, 83, 96, 112, 138, 151, 180, 200
10	Desulphosinigrin	4.654	$C_{10}H_{17}NO_6S$	279	279.077658		60, 73, 85, 103, 127, 145, 163, 213, 262
11	Glycyl-dl-serine	4.763	$C_5H_{10}N_2O_4$	162	162064056		60, 74, 85, 114, 126
12	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	4.929	$C_6H_8O_3$	128	128.047344		57, 72, 85, 94,128

Table 1. Contd.

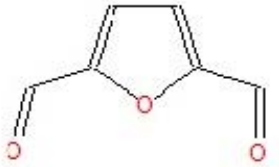
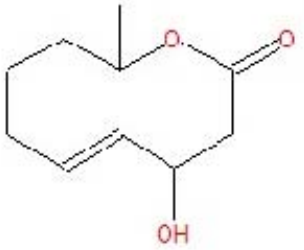
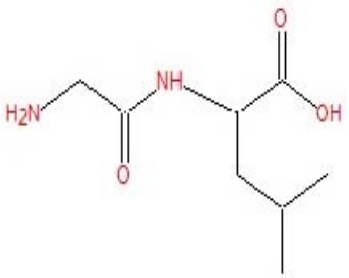
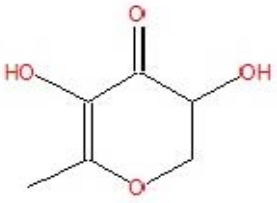
13	2,5-Furandicarboxaldehyde	5.066	$C_6H_4O_3$	124	124.016044		53, 67, 95, 124
14	2H-Oxecin-2-one,3,4,7,8,9,10-hexahydro-4-hydroxy-10-methyl-,[4	5.261	$C_{10}H_{16}O_3$	184	184.109944		55, 70, 81, 95, 112, 124, 142, 166, 184
16	DL-Leucine , N-glycyl	5.616	$C_8H_{16}N_2O_3$	188	188.116093		55, 86, 114, 132, 157, 188
18	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	5.942	$C_6H_8O_4$	144	144.042258		55, 72, 85, 101, 115, 144

Table 1. Contd.

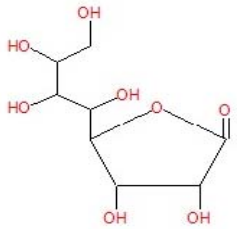
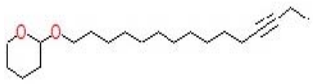
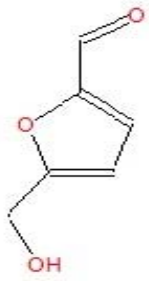
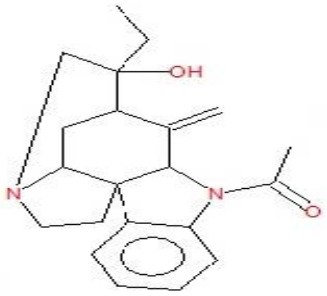
19	l-Gala-l-ido-octonic lactone	6.577	$C_8H_{14}O_8$	238	238.068868		61, 73, 84,112, 127, 142, 159, 189, 220
20	2H-Pyran,tetrahydro-2-(12-pentadecynyloxy)	6.737	$C_{20}H_{36}O_2$	308	308.27153		55, 67, 85, 101, 135, 156, 177, 198, 219, 255, 279, 307
21	5-Hydroxymethylfurfural	7.120	$C_6H_6O_3$	126	126.031694		53, 69, 81, 97, 126
22	Strychane,1-acetyl-20α-hydroxy-16-methylene	8.053	$C_{21}H_{26}N_2O_2$	338	338.199429		57, 70, 88, 97, 130, 166, 196, 224, 239, 253, 281, 295, 338

Table 1. Contd.

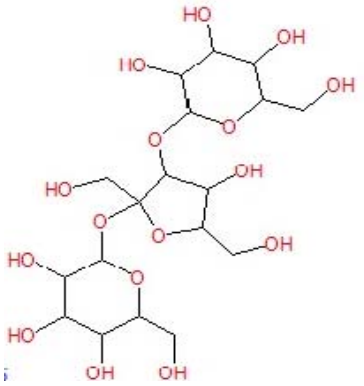
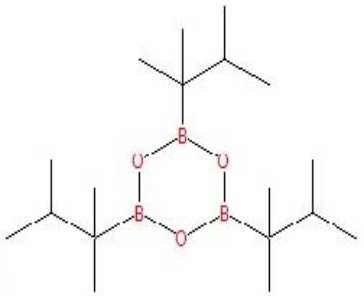
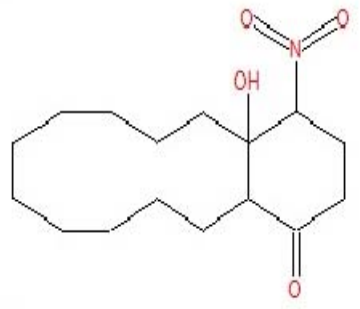
23	α-D-Glucopyranoside ,O-α-D-glucopyranosyl-(1.fwdarw.3)ß-D-fru	7.836		$C_{18}H_{32}O_{16}$	504	504.169035		60, 73, 85, 97,113, 126, 145, 187
24	Boroxin , tris(2,3-dimethylbut-2-yl)	8.442	$C_{18}H_{39}B_3O_3$		336	336.317837		55, 69, 84, 95, 115, 137, 157, 181, 209, 251, 292, 321
25	16-Nitrobicyclo[10.4.0]hexadecane-1-ol-13-one	8.797	$C_{16}H_{27}NO_4^-$		297	297.194008		55, 69, 81, 98, 126, 158, 173, 209, 249, 267, 297

Table 1. Cont'd.

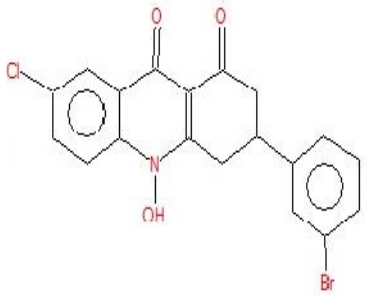
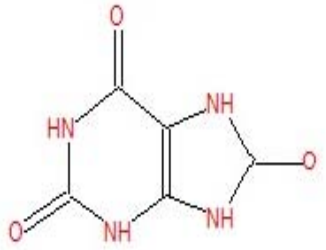
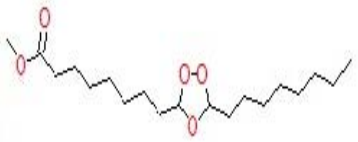
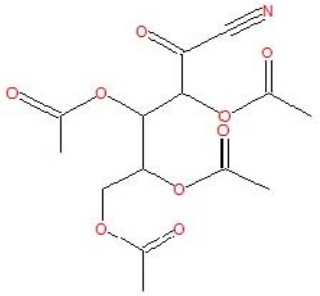
26	3-[3-Bromophenyl]-7-chloro-3,4-dihydro-10-hydroxy-1,9(2H,10H)-a	9.043	$C_{19}H_{13}BrClNO_3$	416	416.976732		57, 69, 97, 147, 219, 246, 321, 401, 419
27	Uric acid	9.672	$C_5H_4N_4O_3$	168	168.02834		54, 69, 82, 97, 125, 140, 168
28	1,2,4-Trioxolane-2-octanoic acid ,5-octyl-,methyl ester	11.320	$C_{19}H_{36}O_5$	344	344.256275		56, 69, 83, 98, 111, 143, 155, 185, 215, 259, 281, 311
29	Tetraacetyl-d-xylonic nitrile	12.276	$C_{14}H_{17}NO_9$	343	343.090332		60, 73, 112, 133, 164, 197, 228, 270

Table 1. Cont'd.

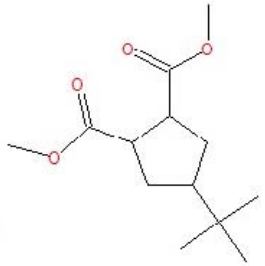
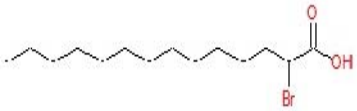
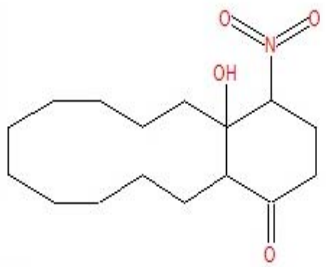
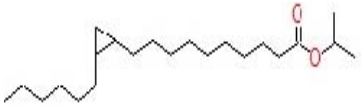
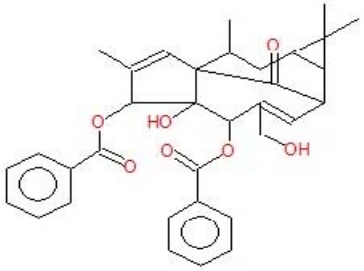
30	1,2-Cyclopentanedicarboxylic acid ,4-(1,1-dimethylethyl)-,dimethyl	13.975		$C_{13}H_{22}O_4$	242	242.151809		57, 67, 81, 107, 126, 154, 186, 211, 242
31	2-Bromotetradecanoic acid	14.771	$C_{14}H_{27}BrO_2$		306	306.119442		55, 73, 83, 99, 111, 138, 153, 201, 227, 249, 306
32	16-Nitrobicyclo[10.4.0]hexadecane-i-1-ol-13-one	14.908	$C_{16}H_{27}NO_4$		297	297.194008		55, 69, 81, 98, 126, 158, 173, 209, 221, 249, 267, 297
33	i-Propyl 11,12-methylene-octadecanoate	15.022		$C_{22}H_{42}O_2$	338	338.318481		55, 69, 83, 97, 111, 125, 139, 167, 193, 236, 259, 279, 296, 338
34	1H-2,8a-Methanocyclopenta[a]cyclopropa[e]cyclodecan-11-one	17.214		$C_{34}H_{36}O_7$	556	556.246105		51, 77, 91, 105, 122, 135, 161, 187, 215, 241, 284, 312, 347

Table 1. Cont'd.

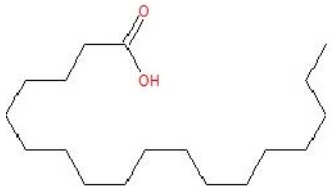
35	Octadecanoic acid	17.048	$C_{18}H_{36}O_2$	284	284.27153		60, 73, 83, 97, 115, 129, 143, 157, 171, 185, 199, 227, 241 ,255, 284

Table 2. Fourier-transorm infrared spectroscopy peak values of *A. niger*.

S/N	Peak (Wave number cm-^{l})	Intensity	Bond	Functional group assignment	Group frequency
1	696.30	58.479	C-H	Aromatic rings	690-900
2	744.52	68.028	C-H	Alkenes	675-995
3	821.68	75.498	C-H	Alkenes	675-995
4	844.82	74.141	C-H	Alkenes	675-995
5	900.76	71.557	C-H	Alkenes	675-995
6	931.62	69.887	C-H	Alkenes	675-995
7	1026.13	52.098	C-F stretch	Aliphatic fluoro compounds	1000-10150
8	1145.72	65.416	C-F stretch	Aliphatic fluoro compounds	1000-10150
9	1207.44	75.125	C-H	Tetiary amine, C-N stretch	1150-1207
10	1234.44	74.798	-	Unknown	-
11	1261.45	75.761	-	Unknown	-
12	1315.45	75.890	-	Aromatic nitro compounds	1310-1390
13	1359.82	74.081	-	Aromatic nitro compounds	1310-1390
14	1377.17	73.205	-	Aromatic nitro compounds	1310-1390
15	1413.82	74.198	-	Ammonium ions	1390-1430
16	1452.40	73.841	-CH3	Methyl-CH. asym	1430-1470
17	1631.78	76.752	-	Organic nitrate	1620-1640
18	1741.72	77.128	-	Unknow	-
19	2852.72	78.925	-	Methylene-CH. asym	2840-2860
20	2924.09	72.033	-	Methylene-CH. asym	2915-2935
21	3118.90	87.299	-	Unknown	-
22	3217.27	83.936	O-H	Normal polymeric O-H stretch	3200-3400
23	3271.27	82.140	O-H	Normal polymeric O-H stretch	3200-3400

Table 3. Zone of inhibition (mm) of test bacterial strains to *A. niger* bioactive compounds and standard antibiotics.

Fungal products Antibiotics	Bacteria				
	Klebsiella pneumonia	***Pseudomonas eurogenosa***	***Staphylococcus aureus***	***Proteus mirabilis***	***Escherichia coli***
Fungal products	6.52±0.61	4.71±0.52	6.16±0.42	5.51±0.62	6.30±0.43
Rifambin	1.12±0.1	1.10±0.1	1.21±0.5	0.60±0.1	0.81±0.2
Streptomycin	1.25±0.3	1.11±0.3	1.30±0.5	1.73±0.2	1.34±0.6
Kanamycin	0.82±0.3	0.53±0.4	0.60±0.2	0.46±0.1	0.92±0.1
Cefotoxime	1.29±0.5	1.50±0.1	1.27±0.1	1.22±0.6	1.25±0.3

Table 4. Zone of inhibition (mm) of test different bioactive compounds and standard antibiotics of plants to *A. niger*.

S/N	Plant	Zone of inhibition (mm)
1	*Nerium olender* (Alkaloids)	4.19±0.25
2	*Ricinus communis* (Alkaloids)	4.70
3	*Datura stramonium* (Alkaloids)	7.81±0.61
4	*Linum usitatissimum* (Crude)	7.60±0.50
5	*Anastatica hierochuntica* (Crude)	3.52±0.09
6	*Gramineae poaceae* (Crude)	7.50±0.13
7	Amphotericin B	5.0±0.20
8	Fluconazol	13.0±0.00
9	Control	0.00

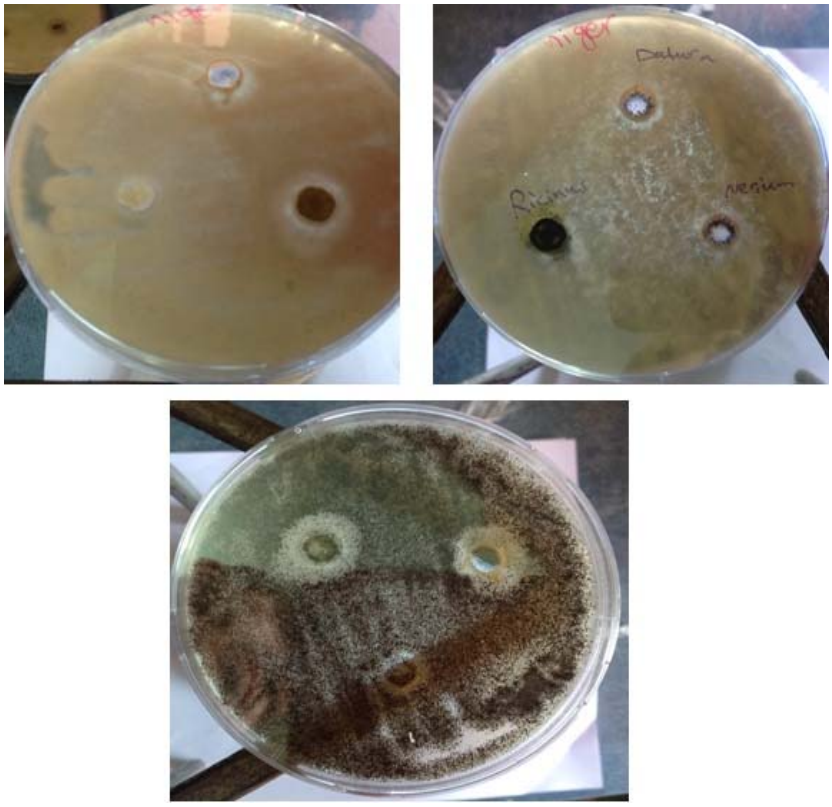


Figure 37. Antifungal activity of extract plant on *A. niger.*

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 *A. niger* can be useful.

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Conflict of interest

Authors have none to declare.

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