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BIOLOGICAL AND GAS CHROMATOGRAPHY MASS SPECTROMETRY STUDIES ON EXTRACT AND FRACTIONS OF *IPHIONIA GRANTIOIDES*

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Abstract

In the health system, the demand for different plants as medicinal plants, has achieved key importance all over the world in the case of both plants and humans. In this research study, the biological activities for instance Antileishmanial, antibacterial, antifungal, anticancer (HeLa, 3T3, and PC3 cell lines), anti-inflammatory, and Brine shrimp lethality assay and Gas chromatography mass spectrometry are studied. Antileishmanial bioassay, microplate alamar blue assay, agar tube dilution method, MTT assay, oxidative burst assay using chemiluminescence technique, β-hatching techniques and Triple quadrupole acquisition method MS parameters are the methods used for Antileishmanial, antibacterial, antifungal, anticancer, anti-inflammatory, Brine shrimp lethality assay and Gas chromatography mass spectrometry. one extract for instance whole plant methanol extract of Iphionia grantioides (WMEIG) and two fractions such as whole plant n-hexane fraction of Iphionia grantioides (WHFIG), whole plant aqueous fraction of Iphionia grantioides (WAFIG) extracted and fractionated from the medicinal plant of Baluchistan, Iphionia grantioides. None of the extracts and fractions of Iphionia grantioides showed Antileishmanial activities and having IC50 above 100. The extract and fractions of Iphionia grantioides were inactive against bacterial strains. All extract and fractions of Iphionia grantioides exhibited no antifungal activities none of the extract and fractions of Iphionia grantioides showed anticancer activities against HeLa, PC3 and 3T3 cell

lines. None of the extracts and fractions of Iphionia grantioides showed anti-inflammatory activities and lethality. GC-MS studies revealed that whole plant methanol extract of Iphionia grantioides (WMEIG) consists of 16 compounds, whole plant n-hexane fraction of Iphionia grantioides (WHFIG) consists of 13 compounds, and whole plant aqueous fraction of Iphionia grantioides (WAFIG) consists of 25 compounds.

Keywords: Iphionia grantioides, Extraction, Fractionation, Antileishmanial, Antibacterial, Antifungal, Anticancer, Anti-inflammatory, BSLA, GC-MS

1-Introduction

The genus iphonia grantioides belong to the family asteraceae. Fleshy leaves are 0.8-1.5cm long, acute-apically 3-lobed, cuneate-linear, or oblanceolate. A single terminal radiate with a diameter of 2-3 cm makes up the capitula. Bracts linear-oblong, herbaceous outer bracts, 5-8 0.8-1.5 m, inner-membranous, linear, acuminate bracts. Linear-oblong bracts, herbaceous outer bracts, and inner-membranous, linear, acuminate bracts make up the involucre, which is 8-12 (-14) mm long, seriate, and thick glandular. The florets are neuter, ligulate, and have 2-3 teeth on the margins. Pappus with just bristles, 2-3 seriate, 22-72 in number, 6-8 mm long, golden-yellow tint. Center florets of hermaphrodites with glandular tips (Naveed et al., 2019).

Hairy, densely coated with glandular trichomes, and nonglandular on both surfaces, the succulent leaves of Iphionia grantioides are hairy, thickly covered with glandular trichomes, and nonglandular on both surfaces. With stomata on both epidermises and anomocyte-type stomatal machinery, the leaf is amphistomatic. Anatomical investigations demonstrate waxy cuticles on both the top and lower epidermises, as well as multicellular non-glandular and glandular trichomes. Trichphyton longitudes, Microsporum canis, candida albicans, and candida glaberata), two human pathogenic fungi (Aspergillus flavus, Aspergillus niger), and three plant pathogenic types (Fusarium Solani, Fusarium Solani) were tested for antifungal activity using crude ethanoic extracts of Iphionia grantioides. (Flower, in the same way, crude extracts of stems, leaves, and roots (500 ug/ml) inhibited fungal strains in the following order: leaves (43.6%) > stem (35%) > root (43.6%) (30 percent). People who have been bitten by a snake are given the plant after it has been cooked in water. Fresh plant paste is applied to wounds to speed up healing. When the leaves are stepped, they are used as an asthma treatment. It's also used as camel fodder and as a washing-machine soup (Naveed et al., 2016).

2 Method and material

2.1 plant material

In this research study, whole plant of iphonia grantioides was used.

2.2 extraction

Iphionia grantioides (Boiss) was dried in its entirety for a month in the shade. This is caused by solar radiation, which eliminates bioactive chemicals found throughout the entire Iphionia grantioides plant (Boiss). Iphionia grantioides (Boiss) was dried and the whole plant was ground into a powder using a mechanical grinder. The 20kg plant powdered was then macerated in 20 L of methanol for just one week, and The mixture was then concentrated at reduced pressure and below 55 °C while being filtered using Whatman filter paper No. 1. There were 600 g of this whole plant crude methanol extract (WMEIG) (Achakzai et al., 2019; Achakzai et al., 2020; Achakzai et al., 2020).

2.3 Fractionation of crude extract

The whole plant n-hexane fraction of Iphionia grantioides was created by fractionating the crude whole plant extract using two solvents, such as aqueous and n-hexane (WHFIG) and whole plant

aqueous fraction of Iphionia grantioides (WAFIG), each weighing 27.72 g and 440 g, respectively(Achakzai et al., 2019; Achakzai et al., 2020; Achakzai et al., 2020)..

2.4 Antileishmanial Activity

Utilizing microplates for culture, the fractions and extracts of medicinal herbs were assessed against Leishmania major (promastigotes). Leishmania major promastigotes (MHOM/Pk/88/DESTO) were cultivated using bulk normal physiological saline in NNN biphasic medium. The mixture was then concentrated at reduced pressure and below 55 °C while being filtered using Whatman filter paper No. 1. There were 37.1 g of this whole plant crude methanol extract (WMEIG). The Leishmania parasites were centrifuged for 10 minutes at 2000 rpm after the promastigotes were extracted at log phase. At the same speed and timing, Leishmania major was washed three times in saline. 180 uL of culture medium were added to each of the 96 wells of a microtitre plate. Fractions and extracts of therapeutic plants were dissolved in PBS with a pH of 7.4 and 0.5% DMSO and 0.5% MeOH to create a 1000 mg/mL stock solution. In order to create a working solution with a concentration range of 1-100 ug/mL, for the fractions and extracts with a 20 ul concentration were added to wells and serially diluted. 100 uL of the parasite culture were given to each well. In this experiment, two rows remained: one for the positive control, which received typical Antileishmanial drugs like amphotericin B (Fluka) and pentamidine, and the other for the negative control, which received medium (ICN). 96-well microtitre plate was incubated for 72 hours at 21- 220C. The program Ezfit 5.03 was used to determine the IC50 values of fractions and extracts with 41 various Antileishmanial activities, such as numbers of motile cells counted on and improved Neubaur counting chamber (Percella Scientific, USA). Examining the culture for signs of cell vitality, such as (Atta-ur-Rahman, 2001).

2.5 Antibacterial activity

Five bacterial strains are used in this research study for instance Salmonella typhi ATCC14028, Pseudomonas aeruginosa ATCC 10145, Staphylococcus aureus NCTC 6571, Bacillus subtilis ATCC 23857 and Escherichia coli ATCC 25922.

Microplate Alamar Blue Assay

The antibacterial activity is assessed using this test. The growing medium for organisms was Mueller Hinton medium. A McFarland turbidity index with a value of 0.5 was employed to modify the inoculums. By dissolving extracts in DMSO, the stock solution was created. media was moved to each well. Except for the control well, where extracts were not added, wells had extracts added to them. Make the wells 200 uL in size. Last but not least, 5x106 cells were added to both the test and control 96-well plates. The plate was sealed with parafilm with the aid of pafilm before being placed in an incubator for 18–20 hours. Alamar Blue Dye was applied to each well, and the plate was shaken for two to three hours at an RPM of 80. The color of the dye, for example, Alamar blue dye was turned pink to demonstrate the development of bacteria. The absorbance at 570 nm was measured with an ELISA reader (Pettit et al., 2005).

2.6 Antifungal Assay

Seven fungi are used in this research study for instance Aspergillus fumigatus, Candida glabarata, Fusarium lini, Microsporum canis, Aspergillus niger, Trichphyton rubrum and Candida albicans.

Agar tube dilution method

It was done by dilution of agar tubes to assess the extracts' antifungal efficacy. Extracts were dissolved in 1 ml of DMSO at a concentration of 24 mg (Merck). In 500 cc of distilled water Sabouraud, 4% glucose agar, and 4 grams of agar-agar were dissolved to create SDA. (SigmaAldrich, Germany). Through a magnetic stirrer, mixture was then carefully stirred. This growth media was steamed until it totally dissolved, then 4ml was added to screw-cap tubes, which were 42 and subsequently autoclaved for 15 minutes at 121oC. After the tubes had cooled to 15 ^{o}C and the SDA had not yet solidified, 66.6 ul of crude extract was placed into it. At room temperature,

tubes were allowed to harden in a slanting orientation. A 4mm-diameter portion of the fungus was injected into the tubes. In other media, the negative control and the positive control, such as standard antifungal medicines, such as DMSO, were utilized. For 3–7 days, tubes were incubated at 27–29 ºC. Fungal cultures were checked twice a week while they were incubating (Atta-ur-Rahman, 2001; Choudhary, 1995).

2.7 Anti-Inflammatory Assay

Oxidative burst assay using chemiluminescence technique

This approach uses 25ul of whole blood diluted in HBSS++ with calcium and magnesium chloride. (St. Louis, USA; Sigma) as well as the fractions and extracts of medicinal plants at a concentration of 25 uL were incubated at 37oC for 15 min 96-well plates were then used to plate this mixture (Costar, NY, USA). The unfinished wells were filled with HBSS++. Cells and HBSS++ were introduced to the control wells. 25 ul each of serum opsonized zymosan and luminol from the St. Louis-based Sigma Chemical Co., Missouri, the United States, were warmly received (Sigma Chemical Co., St. Louis, MO, USA). Relative light units were used to measure the ROS concentration in luminometers. Ibuprofen was used as a common treatment and had an IC50 of 11.2 1.9. (Helfand et al., 1982).

2.8 Brine Shrimp Lethality Assay

B-hatching techniques

B-Hatching was used to separate 50 mg of brine shrimp eggs and incubate them there for two days at 37°C on a hatching tray with filtered brine solution. 2 mL of a solvent were dissolved. Such as methanol, in 20 mg of plant extracts and fractions. The concentration was increased to 10, 100, and 1000ug/mL by dividing this solution into three vials and adding 5, 50, and 500ul to each. The solvent evaporated overnight. Each container received 30 larvae by Pasteur pipette addition. Seawater (5 ml) was added. Twenty-four hours of incubation at a temperature of 25–27oC with light. Other vials included solvent-reference cytotoxic medication along with negative and positive 43 controls. Etoposide, 7.4625ug/mL, was the standard drug utilized in this investigation. The Finney computer programmer was used to calculate the LD50 (Kivack, 2001;Carballo, 2000).

2.9 MTT Assay (PC 3, 3T3 and HeLa cell lines)

The American Type Culture Collection was used to purchase the cell lines of PC 3, 3T3, and HeLa (ATCC). A medium resembling Dulbecco's Eagle modified media was used. It contained 10% FBS, 2% drugs such as penicillin and streptomycin at 100 IU/mL and 100 ug/mL, respectively, maintained in 5% CO2 and incubated at 37 oC. in this assay to create a culture of cancer cell line. Cell lines were extracted once confluence had formed. In a 96-well flat, 5 104 cells were seeded each well. Extracts and fractions containing 50ug/ml were added after 24 hours. Cell lines and a sample were grown in a 96-well plate for 48 hours. Fractions and extracts containing 50 ug/ml were added after 24 hours. Cell lines and a sample were grown in a 96-well plate for 48 hours. The decrease of MTT led to the formation of formazan crystals. Crystals were dissolved by adding 100ul of DMSO, and then, the absorbance at 570 nm was measured in a microplate reader before the measurement (Spectra Max plus, Molecular Devices, USA, CA). Doxorubicin and cyclohexamide were utilized as the standard treatments for HeLa, PC 3, and 3T3 cell lines, respectively. To calculate the % inhibition or reduction in viable cells, the following formula was used: In order to calculate the IC50, a stock solution of the fraction or extracts at a concentration of 20 mM is diluted into a working solution at a concentration of 50 uM. The working solution is then serially diluted to produce less than 50% inhibition. By the aid of the EZ-fit5 program, The IC50 is determined (Mosmann,1983).

2.10 Gas chromatography mass spectrometry (GC-MS) analysis triple quadrupole acquisition method MS parameters

We isolated and quantified chemicals from Iphionia grantioides. When there is, a column HP5MS. 2 uL and mass spectrometer of the fraction or extracts were directly fed into the gas chromatograph mod 5973 Network Mass Selective Detector is used. 6890N Network GC System (Agilent Technologies Palo Alto, California) Agilent Technologies, Palo Alto, California (30 m 44 length, 0.25 mm internal diameter, 0.25 um film width). The incorrect variety of helium gas. A split-splitless injector at 250 oC was injected using a 30:1 split ratio. The oven's schedule was as follows: 70°C for three minutes, followed by six minutes at 180°C, five minutes at 280°C, and finally, ten minutes at 290°C. 250 oC was the MSD transfer line's temperature; the MSD quadrupole temperature was 150 oC; The mass spectra were at 70 eV, the ionization temperature was 230 oC, and the scan was successful in the series between 35 and 300 m/z. By contrasting the mass spectra of the fractions or extracts from Iphionia grantioides with those in the NIST 02 and WILEY libraries (Eman et al., 2016).

3. Results and Discussion

In this research study, one extract for instance whole plant methanol extract of Iphionia grantioides (WMEIG) and two fractions such as whole plant n-hexane fraction of Iphionia grantioides (WHFIG), whole plant aqueous fraction of Iphionia grantioides (WAFIG) extracted and fractionated from the medicinal plant of Baluchistan, Iphionia grantioides. None of the extracts and fractions of showed Antileishmanial activities and having IC50 above 100. The standard drug used for Antileishmanial activities are amphotericin B and pentamidine with IC50 value 3.41 ± 0.02 and 4.56 ± 0.01. The Antileishmanial activities of extracts and fractions of whole plant Iphionia grantioides are represented in table 1.

Table 1: Antileishmanial activities of extracts and fractions of whole plant Iphionia grantioides

S.No	Name of extracts/fractions/Std Drugs	IC_{50} (ug/mL) ± S.D.
1	WMEIG	>100
2	WHFIG	>100
3	WAFIG	>100
9	Pentamidine	4.56 ± 0.01
10	Amphotericin B	3.41 ± 0.02

The extract and fractions of *Iphionia grantioides* were inactive against bacterial strains. Ofloxacin was the standard drug used against bacterial strains with percent inhibition 92.54%, 92.41%, 93.05%, 92.68% and 92.37% against *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli* and *Bacillus subtilis*. The antibacterial activities of extracts and fractions of whole plant *Iphionia grantioides* are represented in Table 2

Table 2: Antibacterial activities of extracts and fractions of whole plant *Iphionia grantioides*

S.No	Bacterial Name	WMEIG	WHFIG	WAFIG	Percentage (%) Inhibition of Drugs (Ofloxacin)
		Percentage (%) Inhibition	Percentage (%) Inhibition	Percentage (%) Inhibition	
1	Salmonella typhi ATCC14028	No Inhibition	No Inhibition	No Inhibition	93.05%
2	Pseudomonas aeruginosa ATCC 10145	No Inhibition	No Inhibition	No Inhibition	92.41%
3	Staphylococcus aureus NCTC 6571	No Inhibition	No Inhibition	No Inhibition	92.54%
4	Bacillus subtilis ATCC 23857	No Inhibition	No Inhibition	No Inhibition	92.37%

All extracts and fractions of *Iphionia grantioides* exhibited no antifungal activities. Miconazole and amphotericin B were the standard drugs used against seven strains of fungi for instance, *Microsporum canis, Candida glabarata, Aspergillus Niger*, *Fusarium lini, Aspergillus fumigatus, Candida albicans* and *Trichphyton rubrum*. Extracts and fractions of antifungal activities of whole plant *Iphionia grantioides* are represented in Table 3.

Table 3: Antifungal activities of extracts and fractions of whole plant *Iphionia grantioides*

S.NO	Name of Fungus	Linear Growth (mm) of Sample	Linear Growth (mm) of Control	Std. Drug	Mic (ug/mol)	WMEIG	WHFIG	WAFIG
						% Inhibition	% Inhibition	% Inhibition
1	*Aspergillus fumigatus*	100	100	Amphoteric in B	100	0%	0%	0%
2	*Candida glabarata*	100	100	Miconazole	110.8	0%	0%	0%
3	*Fusarium lini*	100	100	Amphoteric in B	73..25	0%	0%	0%
4	*Microsporu m canis*	100	100	Miconazole	98.4	0%	0%	0%
5	*Aspergillus niger*	100	100	Miconazole	20	0%	0%	0%
6	*Trichphyton rubrum*	100	100	Miconazole	70	0%	0%	0%
7	*Candida albicans*	100	100	Miconazole	110	0%	0%	0%

None of the extract and fractions of *Iphionia grantioides* showed anticancer activities against HeLa cell line. The standard drug, Doxorubicin inhibited the growth of HeLa cell line 100% with IC_{50} 0.9± 0.14. extracts and fractions of anticancer of whole plant *Iphionia grantioides* are represented in table 4.

Table 4: Anticancer activities (HeLa cell line) of extracts and fractions of whole plant *Iphionia grantioides*

S.No.	Extract/Fraction/Std. Drug	Conc. (ug/mL)	% Inhibition/Stimulation	IC_{50} ± S.D.
1	WMEIG	30	9.7	Inactive
2	WHFIG	30	7.1	Inactive
3	WAFIG	30	2.2	Inactive
5	Doxorubicin	30	100	0.9± 0.14

None of the extract and fractions of *Iphionia grantioides* showed anticancer activities against PC3 cell line. The standard drug, Doxorubicin inhibited the growth of HeLa cell line 89.9% with IC_{50} 1.9± 0.14. extracts and fractions of anticancer whole plant *Iphionia grantioides* are represented in Table 5.

Table 5: Anticancer activities (PC3 cell line) of extracts and fractions of whole plant *Iphionia grantioides*

S.No.	Extract/Fraction/Std. Drug	Conc. (ug/mL)	% Inhibition/Stimulation	IC_{50} ± S.D.
1	WMEIG	30	18.9	Inactive
2	WHFIG	30	16.6	Inactive
3	WAFIG	30	7.1	Inactive
5	Doxorubicin	30	89.9	1.9± 0.14

None of the extract and fractions of *Iphionia grantioides* showed anticancer activities against 3T3 cell line. The standard drug Doxorubicin inhibited 96.2% the growth of the cell line with IC_{50} value 0.1± 0.02. extracts and fractions of anticancer activity of whole plant *Iphionia grantioides* are represented in table 6.

Table 6: Anticancer activities (3T3 cell line) of extracts and fractions of whole plant *Iphionia grantioides*

S.No.	Extract/Fraction/Std. Drug	Conc. (ug/mL)	% Inhibition/Stimulation	IC_{50} ± S.D.
1	WMEIG	30	37.9	Inactive
2	WHFIG	30	35.4	Inactive
3	WAFIG	30	29.2	Inactive
5	Doxorubicin	30	96.2	0.1± 0.02

None of the extract and fractions of *Iphionia grantioides* showed anti-inflammatory activities. The standard drug used for anti-inflammatory activity is Ibuprofen with 73.2% inhibition and IC_{50} 11.2±1.4 ug/mL. Extracts and fractions of anti-inflammatory whole plant *Iphionia grantioides* are represented in table 7.

Table 7: Anti-inflammatory activities of extracts and fractions of whole plant *Iphionia grantioides*

S.No	Extract/Fraction/ Std. Drug	Conc. (ug/mL)	% Inhibition/ Stimulation	IC_{50} ± S.D.
1	WMEIG	50	-20.1	-
2	WHFIG	50	12.8	-
3	WAFIG	50	-3.7	-
5	Ibuprofen	25	73.2	11.2± 1.4 ug/mL

None of the extract and fractions of *Iphionia grantioides* showed lethality. The standard drug used for Brine Shrimp Lethality Assay is Ibuprofen with IC_{50}11.2± 1.4 ug/mL. Extracts and fractions of whole plant of brine shrimp lethality bioassay activities of *Iphionia grantioides* are represented in table 8.

Table 8: Brine shrimp lethality bioassay of extracts and fractions of whole plant *Iphionia grantioides*

S.No	Extract and fractions	Dose (ug/mL)	No. of Shrimps	No. of Survivors	% Mortality	LD50 (ug/ml)	STD. Drug	LD_{50} (ug/ml)	% Mortality
1	WMEIG	10	30	26	13.3		Etoposide	7.5	70
		100	30	21	30				
		1000	30	21	30				
2	WHFIG	10	30	30	0		Etoposide	7.5	70
		100	30	29	3.33				
		1000	30	29	3.33				
3	WAFIG	10	30	30	0		Etoposide	7.5	70
		100	30	30	0				
		1000	30	30	0				

GC-MS studies revealed that whole plant methanol extract of *Iphionia grantioides* (WMEIG) consists of 16 compounds for instance, Dodecane, 1-fluoro, 3-Methyl-2-(2-oxopropyl) furan, Heptane, 1,1-Oxybis-, Arabino-Hex-1-Enitol. 1,5-Anhydro-2-deoxy, 2-propenoic acid, Butyl ester, 2-Undpcene, 6-methyl, 2,4,4-Trimethyal-1-pentanol, 1,3-Dioxolane, 2-pentadecyl-, Sydnone, 3,3-tetramethylenedi-, 0xazole, 4,5-dihydro-2,5-dimethyl-, Isobutyl acrylate, 2Propenoic-acid,2-propenyl ester, 1-[(1-Oxo-2-propenyl) oxy}-2,5-pyrrolidinedione, 3-Hexanol, 2,2-dimethyl, Neo-inositol tri-butane boronate, Phthalylsulfathiazole. Chromatogram of whole plant methanol extracts of Iphionia grantioides (WMEIG) is represented in figure 1. Molecular formula, Molecular mass, RT, area and % composition of compounds 1-16 whole plant methanol extract of *Iphionia grantioides* (WMEIG) are shown in tables 9-11. Mass spectra of compounds 1-16 of whole plant

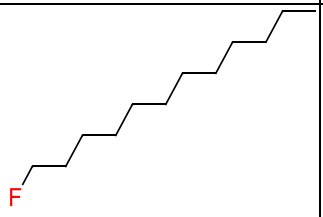
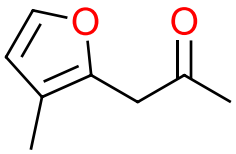
methanol extract of *Iphionia grantioides* (WMEIG) are shown in tables 19-21. Structures and mass spectra of compounds 1-16 are represented in figures 2-17.

Whole plant n-hexane fraction of *Iphionia grantioides* (WHFIG) consists of 13 compounds for instance, Methyl(+-)-2- hydroxyl-3-butenoate, 2,4,4,6,6,8,8-Heptamethyl -1-nonene, N-Mmethyl-3-piperidinecarboxamide, Dodecane, 1-fluoro, Cis-1,2-dihydrocatechol, Octadecane, 1chloro- , Thymol, 1-Decanol, 9-[(trimethyl silyl)oxy]-trifluoroacetate, Cinnamyl acrylate, 12methyloctadec-11-enoic acid trimethyl silyl ester, (Z)-4-methyl-5-(2-oxopropylidene)-5H-Furan2-one,2-Trimethylsiloxy-6-Hexadecenoic acid, methyl ester, 1-Oxaspiro{2,5] octane, 4,4dimethyl-8-methylene. Chromatogram of whole plant n-hexane fraction of Iphionia grantioides (WMEIG) is represented in figure 18. Molecular formula, Molecular mass, RT, area and % composition of compounds 1-13 whole plant n-hexane fraction of *Iphionia grantioides* (WMEIG) are shown in tables 12-14. Mass spectra of compounds 1-13 of whole plant n-hexane fraction of *Iphionia grantioides* (WMEIG) are shown in tables 22 to 24. Structures and mass spectra of compounds 1-13 are represented in figures 19-31.

Whole plant aqueous fraction of *Iphionia grantioides* (WAFIG) consists of 25 compounds for instance, 1-silacyclo-2,4-hexadiene, Cyclopropane, 2-(1,1-dimethyl-2-pentenyl)-1,1-dimethyl, 3methyl-2-(2-oxopropyl) furan, Z, Z-6,28-Heotatriactontadien-2-one, 3-eicosyne, 1Nonylcycloheptane, 3-n-butylthiolane, 1 H-imidazole[1,2-b] pyrazole, 1-methyl-, 5Hydroxymethylfurfural, 1-ethyl-2-hydroxymethylimidazole, 12-methyloctadec-11-enoic acid trimethylsilyl ester, 1,4-Dioxan-2-one, 3,3-dimethyl-5-[(trimethylsilyl)oxy) methyl], Di(1methylcyclobutyl)ether, Heptacosanoic acid, 2,4-dimethoxy-methylester, Isosorbide, 2TBDMS derivative, d-mannitol, 1,1-o-1,16-hexadecanediylbis-,3,7,11,15,18-pentaoxa-2,19- , 7-bromo-1heptanol. TMS derivative, 4,4-Dimethyl-oct-5-enal, 1,3-dioxolane, 4-pentyl-5-propyl-2,2bis(trifluoromethyl)-cis-, 7-ethyl-2-undecen-6-one, 1,2,3,4-Hexadecanetetrol, 2-Butenoic acid, 4,4-dimethoxy- methyl ester, 2-hydroxyoctanoic acid, d-mannitol, 1-O-(22-Hydroxydocosyl).

Chromatogram of the whole plant aqueous fraction of Iphionia grantioides (WMEIG) is represented in Figure 32. Molecular formula, Molecular mass, RT, area and % composition of compounds 1-25 whole plant aqueous fraction of *Iphionia grantioides* (WMEIG) are shown in tables 15-18. Mass spectra of compounds 1-25 of whole plant aqueous fraction of *Iphionia grantioides* (WMEIG) are shown in tables 25-29. Structures and mass spectra of compounds 1-25 are represented in figures 33-56.

Table 9: Molecular formula, Molecular mass, RT, area and % composition of compounds 1-5 whole plant methanol extract of *Iphionia grantioides* (WMEIG)

Compd	Name	Molecular Formula	Molecular Mass	Structure	RT
1	Dodecane, 1-fluoro	$C_{12}H_{25}F$	188		3.544
2	3-Methyl-2-(2oxopropyl) furan	$C_8H_{10}O_2$	138		3.649
3	Heptane, 1,1Oxybis-	$C_{14}H_{30}O$	214		3.714

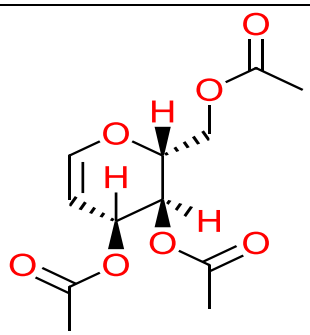
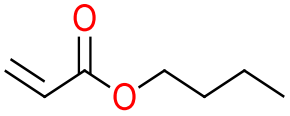
4	Arabino-Hex-1-Enitol. 1,5-Anhydro-2-deoxy	$C_6H_{10}O_4$	146		3.958
5	2-propenoic acid, Butyl ester	$C_7H_{12}O_2$	128		4.139

Table 10: Molecular formula, Molecular mass, RT, area and % composition of compounds 6-10 whole plant methanol extract of *Iphionia grantioides* (WMEIG)

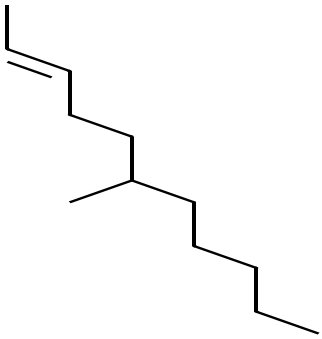
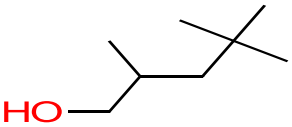
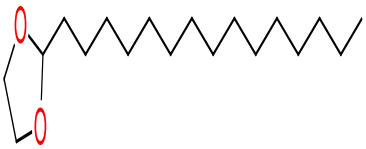
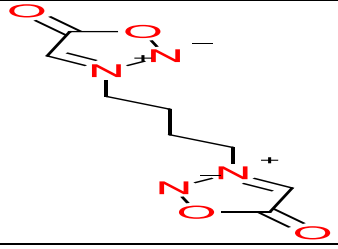
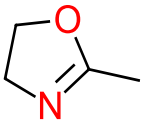
Compd	Name	Molecular Formula	Molecular Mass	Structure	RT
6	2-Undecene, 6methyl	C12H24	168		4.214
7	2,4,4-Trimethyal-1pentanol	C8H18O	130		4.224
8	1,3-Dioxolane, 2pentadecyl-	C18H36O2	284		4.709
9	Sydnone, 3,3tetramethyl enedi-	C8H10N4O4	226		5.93
10	0xazole, 4,5dihydro-2,5dimethyl-	C8H10N4O4	99		5.224

Table 11: Molecular formula, Molecular mass, RT, area and % composition of compounds 11-16 whole plant methanol extract of *Iphionia grantioides* (WMEIG)

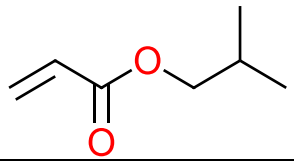
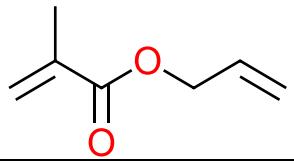
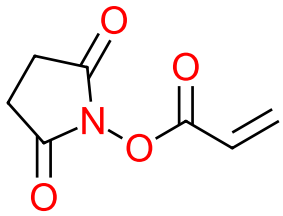
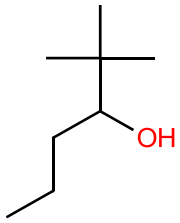
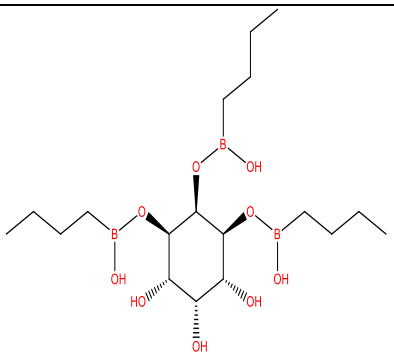
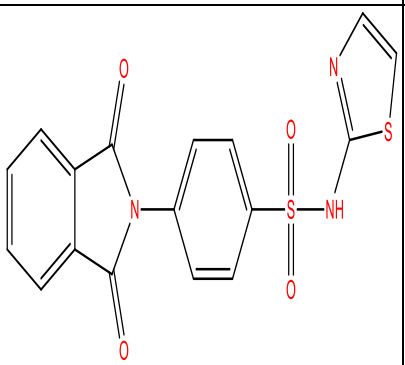
Compd	Name	Molecular Formula	Molecular Mass	Structure	RT
11	Isobutyl acrylate	$C_7H_{12}O_2$	128		5.504
12	2-Propenoicacid,2-propenyl ester	$C_6H_8O_2$	112		5.599
13	1-[(1-Oxo-2propenyl) oxy}-2,5-pyrrolidinedione	$C_7H_7NO_4$	169		5.825
14	3-Hexanol, 2,2dimethyl	$C_8H_{18}O$	130		6.945
15	Neo-inositol tributane boronate	$C_{18}H_{33}B_3O_6$	378		8.741
16	Phthalylsulfathiazole	$C_{17}H_{13}N_3O_5S_2$	403		9.706

Table 12: Molecular formula, Molecular mass, RT, area and % composition of compounds 1-5 whole plant n-hexane fraction of *Iphionia grantioides* (WMEIG)

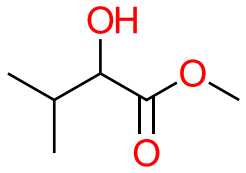
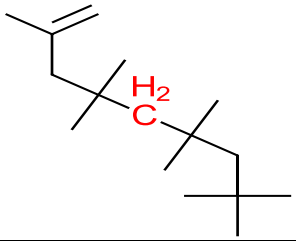
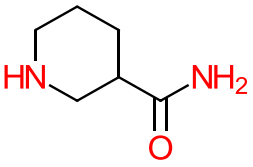
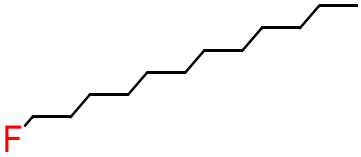
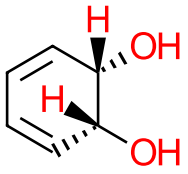
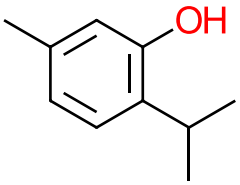
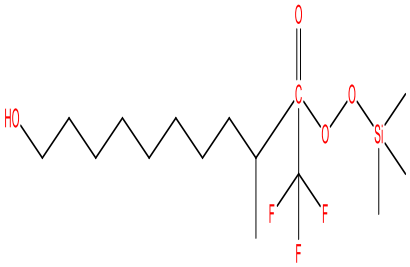
Compd	Name	Molecular Formula	Molecular Mass	Structure	RT
1	Methyl(+-)-2-hydroxyl-3butenoate	$C_5H_8O_3$	116		3.324
2	2,4,4,6,6,8,8-Heptamethyl -1nonene	$C_{16}H_{32}$	224		3.729
3	N-M-methyl-3piperidinecarboxamide	$C_7H_{14}N_2O$	142		3.784
4	Dodecane, 1-fluoro	$C_{12}H_{25}F$	188		3.829
5	Cis-1,2dihydrocatechol	$C_6H_8O_2$	112		4.899

Table 13: Molecular formula, Molecular mass, RT, area and % composition of compounds 6-10 whole plant n-hexane fraction of *Iphionia grantioides* (WMEIG)

Compd	Name	Molecular Formula	Molecular Mass	Structure	RT
6	Octadecane, 1chloro-	$C_{18}H_{37}Cl$	288		7.95
7	Thymol	$C_{10}H_{14}O$	150		9.411
8	1-Decanol, 9[(trimethylsilyl)oxy]trifluoroacetate	$C_{15}H_{29}F_3O_3Si$	342		11.592

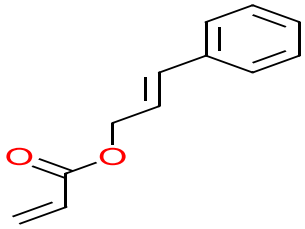
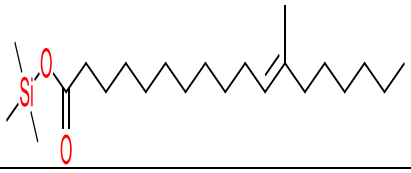
9	Cinnamyl acrylate	$C_{12}H_{12}O_2$	188		12.477
10	12-methyloctadec11-enoic acid trimethyl silyl ester	$C_{22}H_{44}O_2Si$	368		13.788

Table 14: Molecular formula, Molecular mass, RT, area and % composition of compounds 11-13 whole plant n-hexane fraction of *Iphionia grantioides* (WMEIG)

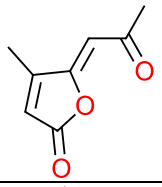
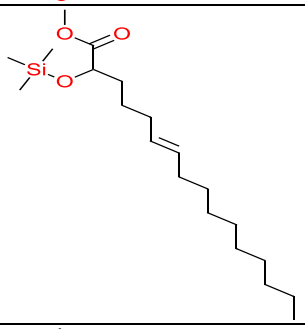
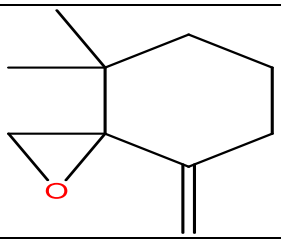
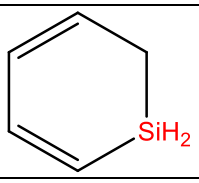
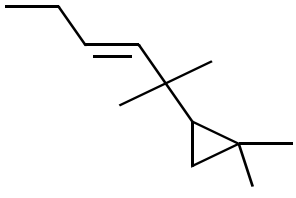
Compd	Name	Molecular Formula	Molecular Mass	Structure	RT
11	(Z)-4-methyl-5-(2oxopropylidene)5H-Furan-2-one	$C_8H_8O_3$	152		13.833
12	2-Trimethylsiloxy6-Hexadecenoic acid, methyl ester	$C_{20}H_{14}O_3Si$	356		13.873
13	1-Oxaspiro{2,5] octane, 4,4dimethyl-8methylene	$C_{10}H_{16}O$	152		14.448

Table 15: Molecular formula, Molecular mass, RT, area and % composition of compounds 1-5 whole plant aqueous fraction of *Iphionia grantioides* (WMEIG)

Compd	Name	Molecular Formula	Molecular Mass	Structure	RT
1	1-silacyclo-2,4hexadiene	C_5H_8Si	96		3.344
2	Cyclopropane, 2(1,1-dimethyl-2pentenyl)-1,1dimeth	$C_{12}H_{22}$	166		3.344

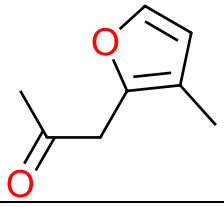
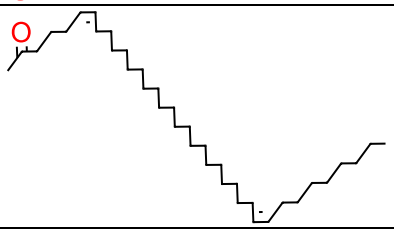
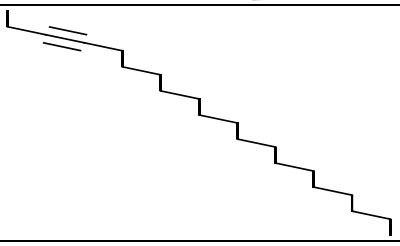
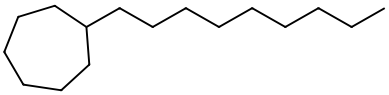
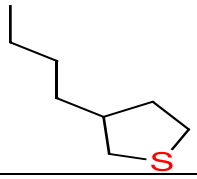
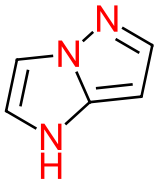
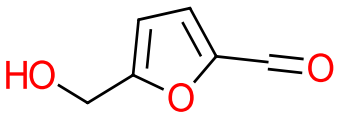
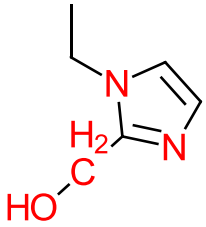
	yl				
3	3-methyl-2-(2oxopropyl)furan	$C_8H_{10}O_2$	138		3.683
4	Z, Z-6,28-Heotatriacontadien -2-one	$C_{37}H_{70}O$	530		3.824
5	3-eicosyne	$C_{20}H_{38}$	278		3.989
6	1-Nonylcycloheptane	$C_{16}H_{32}$	224		5.379
7	3-n-butylthiolane	$C_8H_{16}S$	144		6.19

Table 16: Molecular formula, Molecular mass, RT, area and % composition of compounds 1-5 whole plant aqueous fraction of *Iphionia grantioides* (WMEIG)

Compd	Name	Molecular Formula	Molecular Mass	Structure	RT
8	1 H-imidazol[1,2 b]pyrazole, 1methyl-	$C_6H_7O_2N_3$	121		6.305
9	5-Hydroxymethylfurfural	$C_6H_6O_3$	126		8.611
10	1-ethyl-2hydroxymethylimidazole	$C_6H_{10}N_2O$	126		9.621

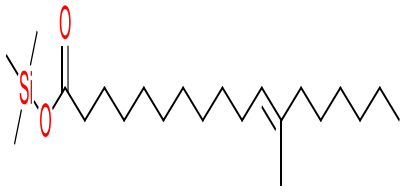
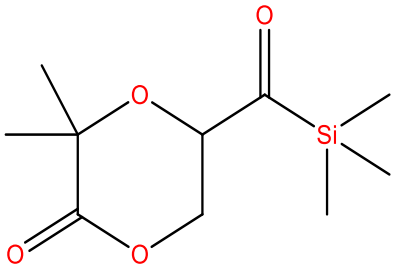
11	12-methyloctadec11-enoic acid trimethylsilyl ester	$C_{22}H_{44}O_2Si$	368		12.077
12	1,4-Dioxan-2-one, 3,3-dimethyl-5[(trimethylsilyl)oxy) methyl]	$C_{10}H_{20}O_4Si$	232		12.142

Table 17: Molecular formula, Molecular mass, RT, area and % composition of compounds 1-5 whole plant aqueous fraction of *Iphionia grantioides* (WMEIG)

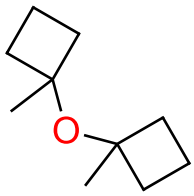
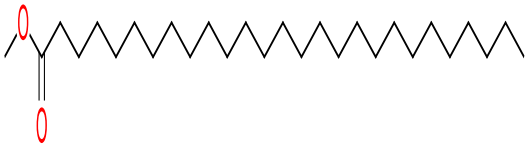
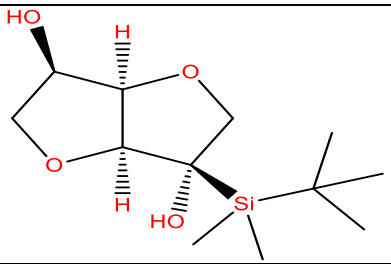
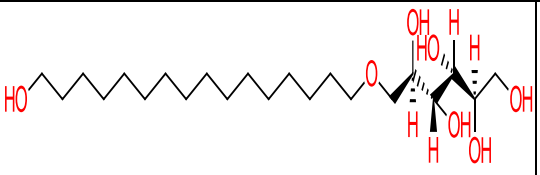
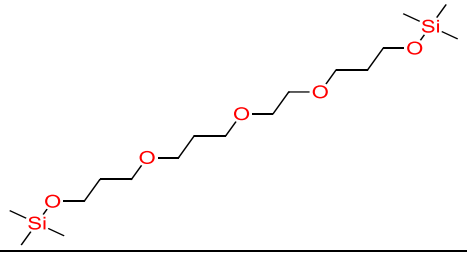
Compd	Name	Molecular Formula	Molecular Mass	Structure	RT
13	Di (1-methylcyclobutyl)ether	$C_{10}H_{18}O$	154		12.242
14	Heptacosanoic acid, 2,4dimethoxymethylester	$C_{30}H_{60}O_4$	484		12.883
15	Isosorbide, 2TBDMS derivative	$C_{18}H_{38}O_4Si_2$	374		12.898
16	d-mannitol, 1,1-o-1,16-hexadecanediylbis-	$C_{28}H_{58}O_{12}$	586		13.068
17	3,7,11,15,18pentaoxa-2,19disilaeicosane,2,2,19,19-tetramethyl	$C_{17}H_{14}O_5Si_2$	380		13.708

Table 18: Molecular formula, Molecular mass, RT, area and % composition of compounds 1-5 whole plant aqueous fraction of *Iphionia grantioides* (WMEIG)

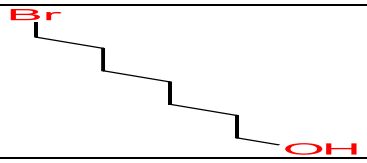
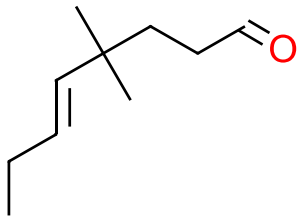
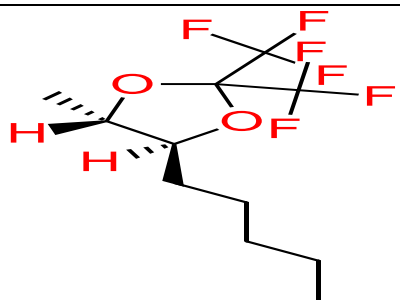
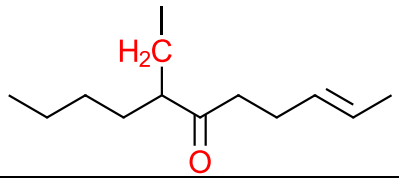
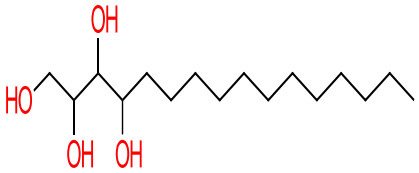
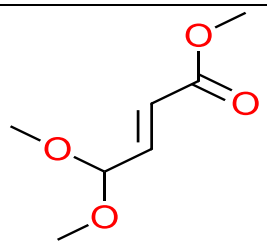
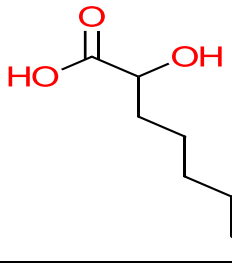
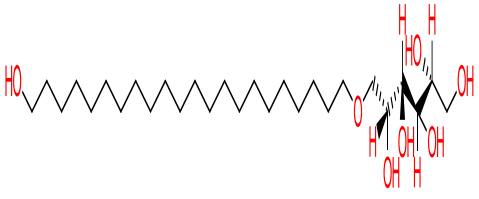
Compd	Name	Molecular Formula	Molecular Mass	Structure	RT
18	7-bromo-1heptanol. TMS derivative	$C_{10}H_{23}BrOSi$	266		14.228
19	4,4-Dimethyl-oct-5-enal	C10H18O	154		14.293
20	1,3-dioxolane, 4pentyl-5-propyl-2,2-bis(trifluoromethyl) -cis-	$C_{13}H_{20}F_6O_2$	322		14.29.3
21	7-ethyl-2-undecen-6-one	$C_{13}H_{24}O$	196		14.463
22	1,2,3,4-Hexadecane tetrol	$C_{16}H_{34}O_4$	290		14.608
23	2-Butenoic acid, 4,4-dimethoxy-methyl ester	$C_7H_{12}O_4$	160		14.688
24	2-hydroxyoctanoic acid	$C_8H_{16}O_3$	160		14.868
25	d-mannitol, 1-O-(22-Hydroxydocosyl)-	$C_{28}H_{58}O_7$	506		14.983

Table 19: Mass spectra of compounds 1-5 of whole plant methanol extract of *Iphionia grantioides* (WMEIG)

Compd	m/z (% Relative abundance)
1	188(M^+), 97(103), 85(154), 84(104), 83(141), 71(382), 70(184), 69(312), 57(999), 56(261), 55(538)
2	138(M^+), 109(240), 95(450), 71(450), 70(250), 69(680), 67(230), 65(210), 57(999), 56(260), 55(770)
3	214(M^+), 98(103), 97(98), 70(223), 69(101), 57(999), 56(157), 55(155), 43(228), 41(188), 29(107)
4	146(M^+), 74(257), 73(999), 71(152), 69(221), 57(191), 56(344), 55(320), 45(154), 43(280), 41(158)
5	128(M^+), 85(41), 73(425), 57(59), 56(540), 55(999), 43(32), 41(140), 29(76), 28(36), 27(168)

Table 20: Mass spectra of compounds 6-10 of whole plant methanol extract of *Iphionia grantioides* (WMEIG)

Compd	m/z (% Relative abundance)
6	168(M^+), 97(226), 71(130), 70(244), 69(407), 57(999), 56(932), 55(692), 43(526), 41(403), 29(265)
7	130(M^+,), 87(301), 73(630), 72(348), 69(268), 57(555), 55(999), 43(381), 41(630), 39(256), 29(225)
8	284(M^+), 283(18), 74(35), 73(999), 69(16), 57(28), 55(33), 45(46), 43(53), 41(39), 29(17)
9	226(M^+), 168(40), 142(10), 141(100), 85(70), 69(70), 68(50), 57(30), 56(330), 55(999), 54(80)
10	99($M^{+\cdot}$,125), 56(159), 55(999), 54(181), 43(190), 42(93), 29(75), 28(181), 27(148), 26(59),

Table 21: Mass spectra of compounds 11-16 of whole plant methanol extract of *Iphionia grantioides* (WMEIG)

Compd	m/z (% Relative abundance)
11	128(M^+,), 85(90), 73(112), 57(51), 56(435), 55(999), 43(49), 41(143), 39(69), 29(66), 27(199)
12	112(M^+), 97(31), 67(50), 57(54), 56(59), 55(999), 41(146), 39(130), 29(27), 27(136), 26(34)
13	169(M^+, 7), 87(16), 70(27), 56(76), 55(999), 43(6), 42(24), 28(84), 27(168), 26(33)
14	130(M^+,), 87(301), 73(630), 72(348), 69(268), 57(555), 55(999), 43(381), 41(630), 39(256), 29(225)
15	378(M^+), 252(229), 239(181), 237(241), 139(355), 126(999), 125(281), 83(348), 70(366), 55(183), 43(177)
16	403(M^+), 191(616), 156(785), 108(590), 104(391), 92(999), 76(412), 65(609), 55(219), 50(229), 45(211)

Table 22: Mass spectra of compounds 1-5 of whole plant n-hexane fraction of *Iphionia grantioides* (WMEIG)

Compd	m/z (% Relative abundance)
1	116(M^+), 87(170), 84(387), 59(186), 57(999), 56(106), 55(163), 31(193), 29(408), 27(174), 15(98)
2	224(M^+), 113(228), 112(77), 97(222), 84(79), 83(117), 69(79), 57(999), 56(83), 55(314), 51(59)
3	142(M^+), 86(567), 84(999), 83(335), 82(276), 68(229), 58(235), 57(690), 56(482), 55(454), 42(159)
4	188(M^+), 97(103), 85(154), 84(104), 83(141), 71(382), 70(184), 69(312), 57(999), 56(261), 55(538),
5	112(M^+,), 94(724), 83(255), 68(208), 66(999), 65(405), 55 (406), 40(236), 39(655), 29(269), 27(197),

Table 23: Mass spectra of compounds 6-10 of whole plant n-hexane fraction of *Iphionia grantioides* (WMEIG

Compd	m/z (% Relative abundance)
6	288(M^+), 91(334), 85(365), 83(209), 71(595), 69(303), 57(999), 55(396), 43(727), 41(352), 29(170)
7	150(M^+,248), 136(89), 135(999), 117(87), 115(91), 91(157), 77(80), 51(56), 41(55), 39(94),
8	342(M^+), 117(534), 103(305), 97(498), 83(999), 75(377), 73(578), 69(550), 57(234), 55(649), 41(168),
9	188($M^{+\cdot}$,221), 133(413), 117(233), 116(219), 115(590), 105(270), 91(113), 77(152), 55(999), 27(210),
10	368(M^+), 357(438), 243(329), 129(300), 117(389), 83(300), 77(609), 75(999), 73(879), 69(558), 55(820)

Table 24: Mass spectra of compounds 11-13 of whole plant n-hexane fraction of *Iphionia grantioides* (WMEIG)

Compd	m/z (% Relative abundance)
11	152($M^{+\cdot}$340), 137(999), 110(410), 109(160), 97(220), 69(960), 68(210), 57(210), 55(270), 53(270),
12	356(M^+), 95(403), 91(443), 89(401), 75(502), 74(335), 73(999), 69(337), 67(347), 57(337), 55(453),
13	152(M^+,), 137(999), 109(420), 107(310), 96(320), 91(300), 81(290), 79(410), 67(300), 43(300), 41(370)

Table 25: Mass spectra of compounds 1-5 of whole plant aqueous fraction of *Iphionia grantioides* (WMEIG)

Compd	m/z (% Relative abundance)
1	96(M^+,999), 95(679), 70(689), 69(330), 68(619), 67(669), 66(350), 55(639), 53(330), 43(709),
2	166($M^{+\cdot}$), 110(536), 96(71), 95(999), 81(169), 69(107), 68(79), 67(171), 55(342), 43(77), 41(233)
3	138($M^{+\cdot}$), 109(240), 95(450), 71(450), 70(250), 69(680), 67(230), 65(210), 56(260), 57(999), 55(770)
4	530($M^{+\cdot}$), 97(560), 95(632), 83(791), 81(725), 71(674), 69(999), 68(559), 67(862), 57(673), 55(965)
5	278(M^+,), 110(552), 109(920), 95(768), 82(835), 81(783), 69(422), 68(557), 67(999), 55(454), 43(503)

Table 26: Mass spectra of compounds 6-10 of whole plant aqueous fraction of *Iphionia grantioides* (WMEIG)

Compd	m/z (% Relative abundance)
6	224($M^{+\cdot}$), 97(389), 96(240), 83(110), 81(229), 69(248), 67(202), 57(103), 56(110), 55(999), 53(80)
7	144($M^{+,830}$), 115(340), 101(999), 95(240), 87(630), 82(240), 81(770), 67(300), 60(590), 55(990),
8	121($M^{+,999}$), 120(470), 106(220), 80(480), 79(210), 54(999), 53(450), 42(140), 40(140), 39(230),
9	126($M^{+,779}$), 125(145), 109(134), 97(999), 69(326), 53(145), 51(115), 41(732), 39(358), 29(171),
10	126($M^{+,721}$), 109(295), 97(824), 81(737), 69(999), 68(356), 54(386), 42(657), 41(567), 40(458),

Table 27: Mass spectra of compounds 11-15 of whole plant aqueous fraction of *Iphionia grantioides* (WMEIG)

Compd	m/z (% Relative abundance)
11	368($M^{+\cdot}$), 353(438), 243(329), 129(300), 117(389), 83(300), 77(609), 75(999), 73(879), 77(609), 55(820),
12	232(M^{+}), 143(236), 131(544), 129(201), 103(475), 101(249), 75(409), 73(809), 69(999), 41(491), 39(172),
13	154($M^{+\cdot}$), 126(40), 98(100), 88(40), 86(45), 70(60), 69(999), 68(80), 67(50), 58(200), 53(20)
14	484($M^{+\cdot}$), 382(253), 381(821), 367(288), 366(999), 161(636), 129(869), 104(669), 71(233), 57(286), 43(253),
15	374($M^{+\cdot}$), 317(184), 185(152), 133(214), 129(202), 117(999), 101(180), 75(400), 73(845), 69(753), 59(207)

Table 28: Mass spectra of compounds 16-20 of whole plant aqueous fraction of *Iphionia grantioides* (WMEIG)

Compd	m/z (% Relative abundance)
16	586(M^{+},) 129(653), 111(438), 97(444), 83(615), 73(906), 69(999), 61(414), 57(512), 55(744) 43(413),
17	380(M^{+}), 131(461), 130(293), 129(178), 117(584), 116(277), 115(154), 103(350), 75(198), 73(999), 58(146)
18	266(M^{+},), 167(115), 139(123), 137(115),103 (272), 97(999), 75(211), 73(364), 69(200), 55(997), 41(112),
19	154($M^{+\cdot}$), 126(168), 97(666), 95(148), 81(168), 69(328), 67(109), 57(90), 55(999), 43(110), 41(326)
20	322(M^{+},), 253(216), 139(161), 97(373), 83(999), 73(121), 69(515), 67(127), 57(237), 56(163), 55(475)

Table 29: Mass spectra of compounds 21-25 of whole plant aqueous fraction of *Iphionia grantioides* (WMEIG)

Compd	m/z (% Relative abundance)
21	196(M^{+}), 97(595), 73(168), 70(144), 69(999), 57(146), 55(779), 53(192), 43(208), 41(400), 39(138),
22	290(M^{+}), 97(108), 83(139), 74(999), 73(152), 69(183), 57(119), 56(264), 55(182), 43(160), 41(138),
23	160(M^{+}), 129(999), 113(110), 101(829), 75(483), 69(248), 59(124), 55(198), 45(171), 29(151), 27(149),
24	160(M^{+}), 115(511), 97(617), 76(379), 69(230), 57(212), 55(999), 45(140), 44(146), 43(579), 41(477)
25	506(M^{+}), 103(394), 97(604), 83(609), 73(999), 71(353), 69(705), 57(583), 55(834), 43(548), 41(398),

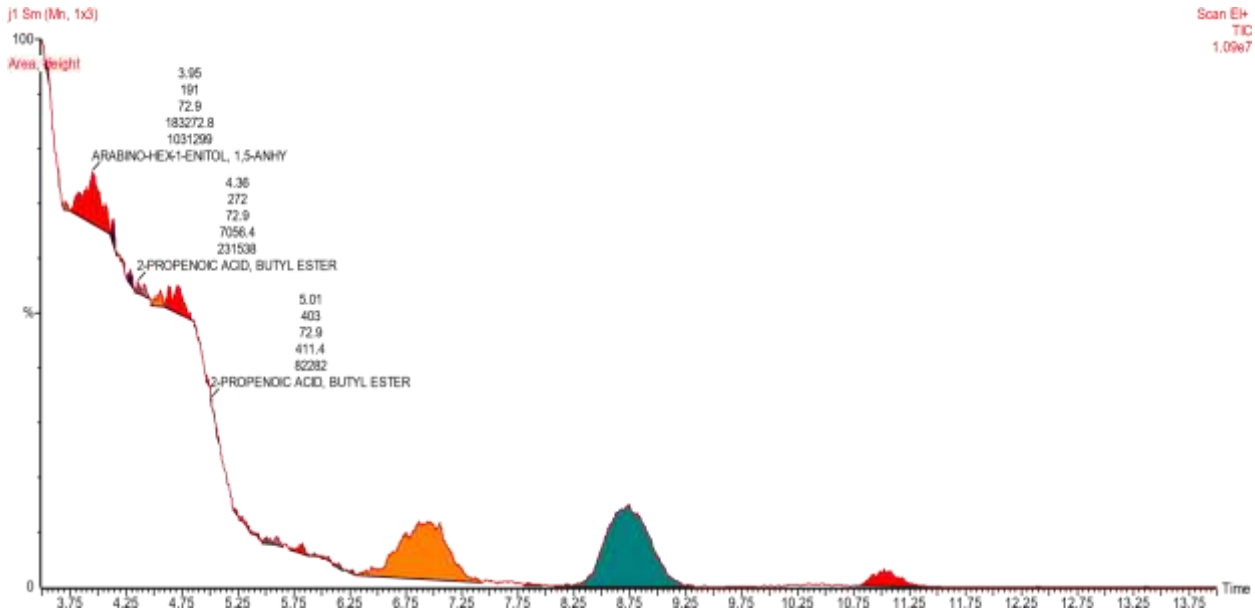


Figure 1: Chromatogram of Whole Plant methanol extract of Iphionia grantioides (WMEIG)

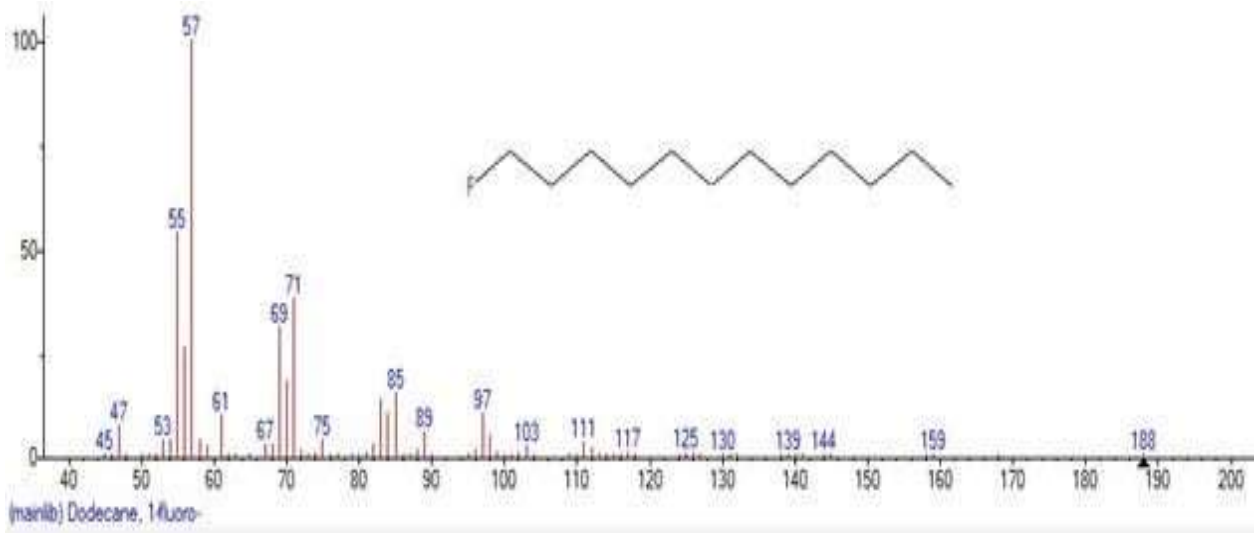


Figure 2: Mass spectra of Dodecane, 1-fluoro of Whole Plant methanol extract of Iphionia grantioides (WMEIG)

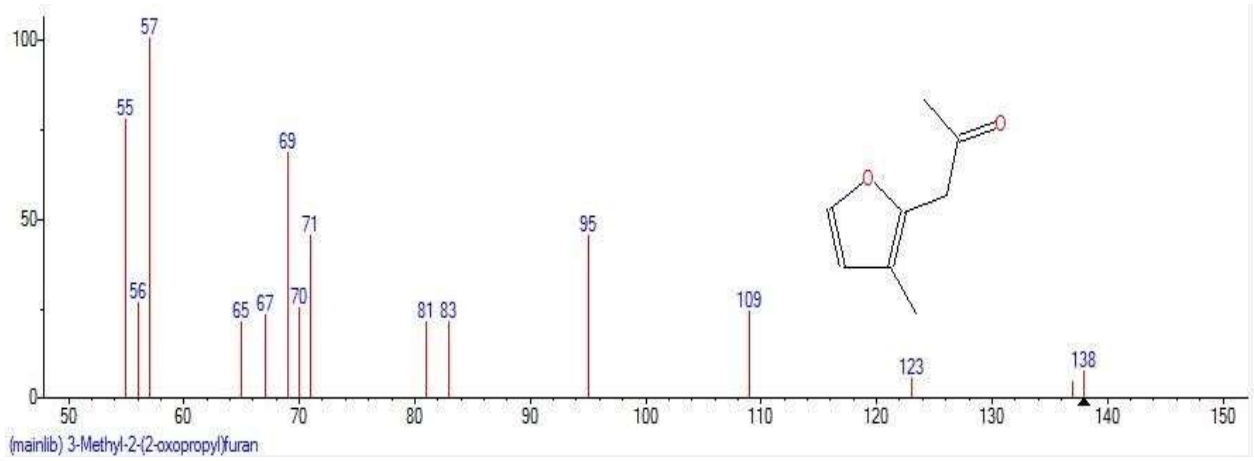


Figure 3: Mass spectra of of 3-Methyl-2-(2-oxopropyl) furan of Whole Plant methanol extract of Iphionia grantioides (WMEIG)

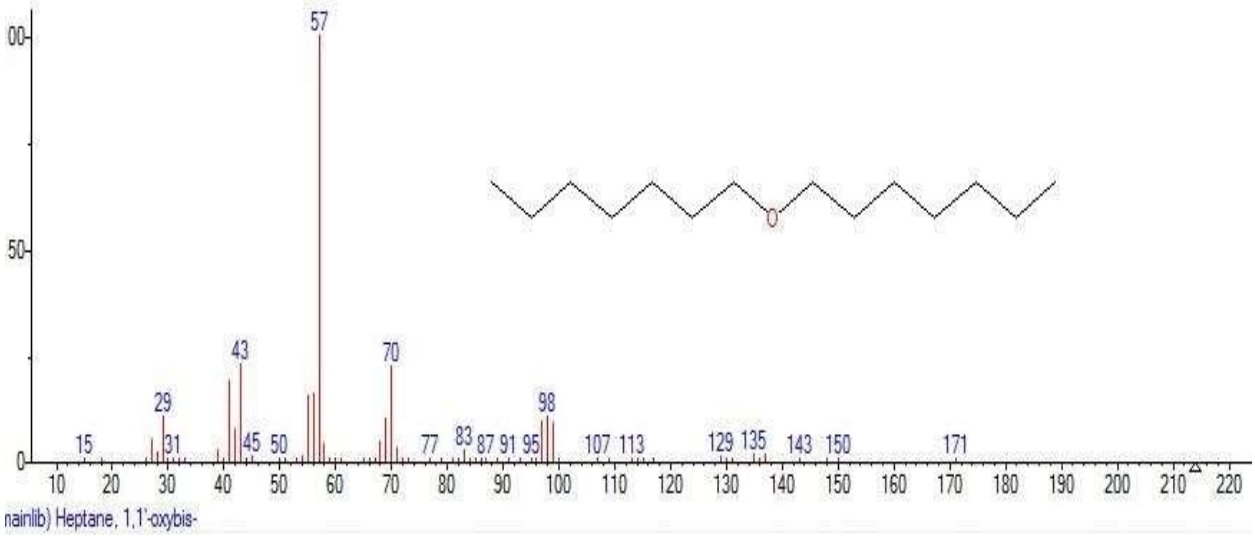


Figure 4: Mass spectra of Heptane, 1,1-Oxybis- of Whole Plant methanol extract of *Iphionia grantioides* (WMEIG)

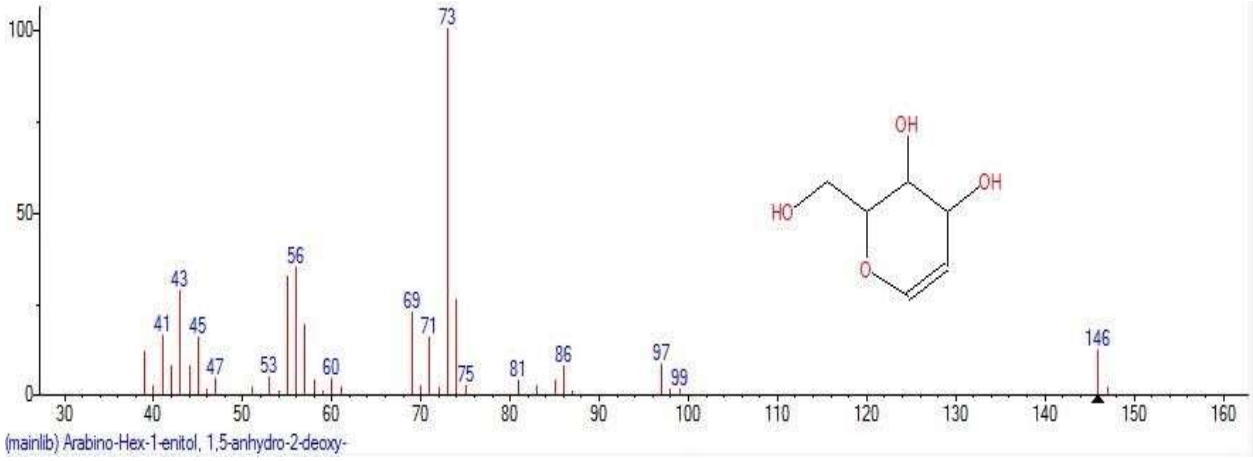


Figure 5: Mass spectra of Arabino-Hex-1-Enitol. 1,5-Anhydro-2-deoxy of Whole Plant methanol extract of Iphionia grantioides (WMEIG)

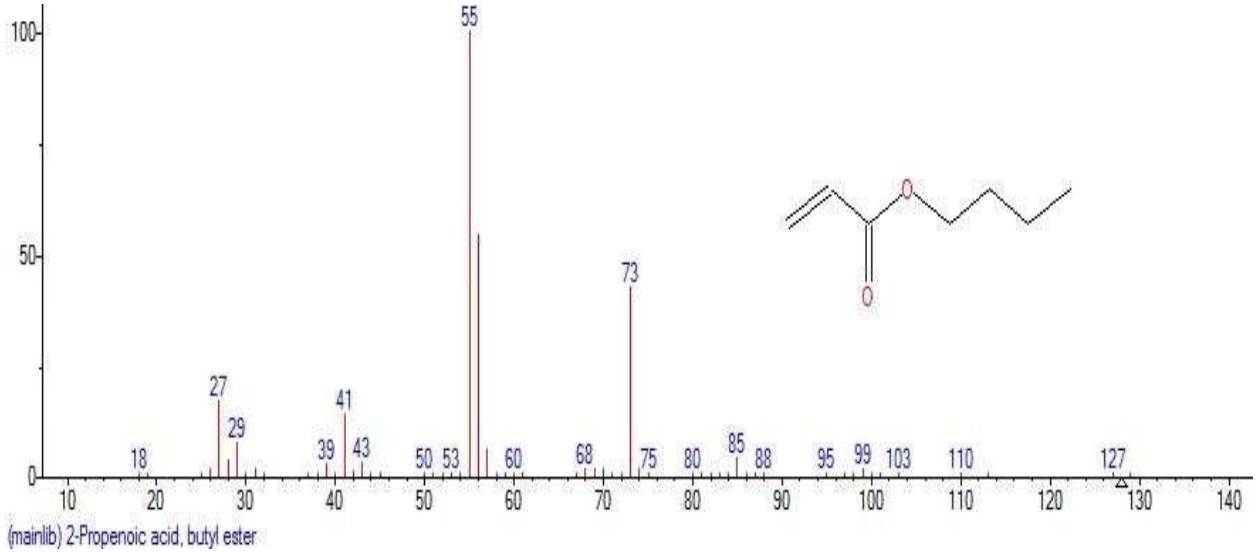


Figure 6: Mass spectra of 2-propenoic acid, Butyl ester of Whole Plant methanol extract of *Iphionia grantioides* (WMEIG

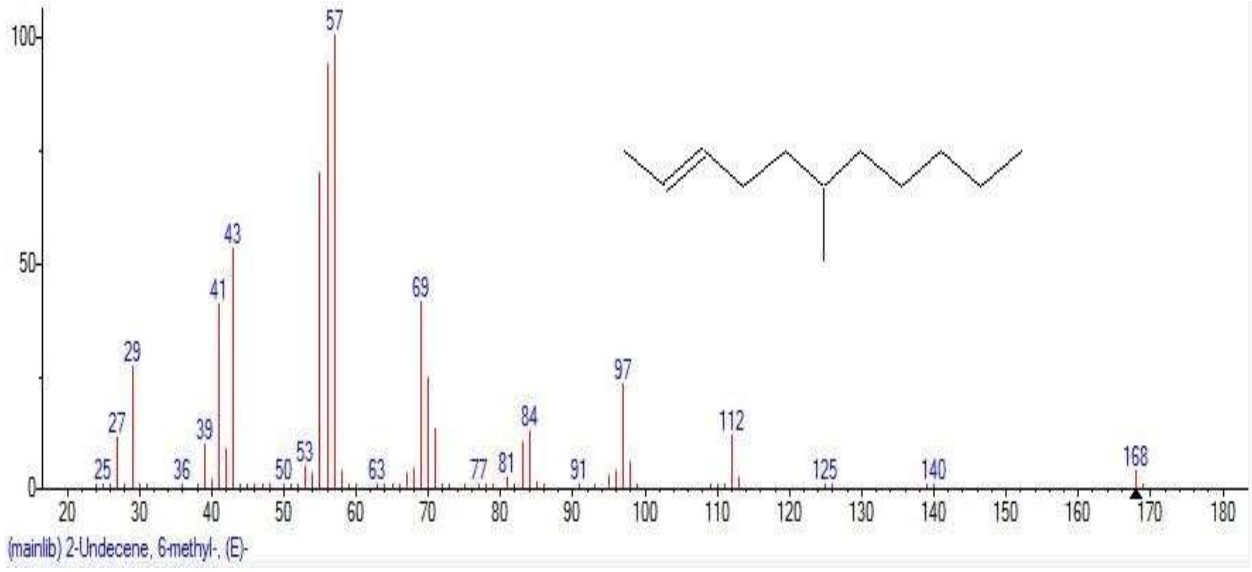


Figure 7: Mass spectra of 2-Undecene, 6-methyl of Whole Plant methanol extract of *Iphionia grantioides* (WMEIG)

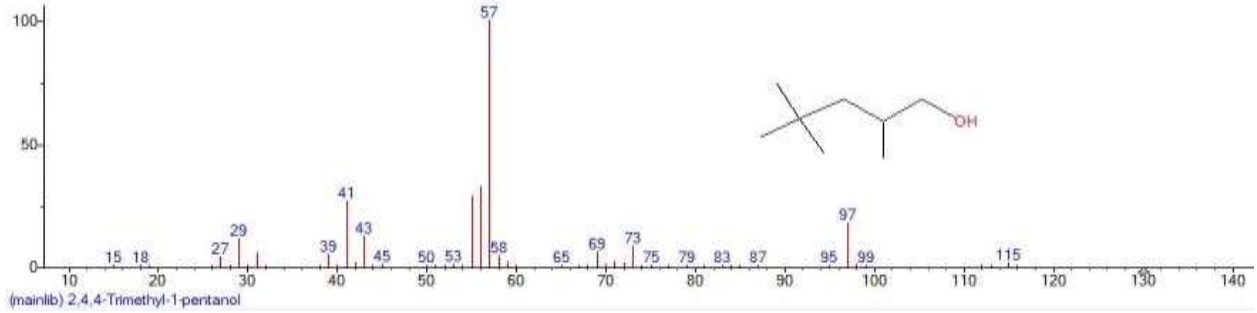


Figure 8: Mass spectra of 2,4,4-Trimethyal-1-pentanol of Whole Plant methanol extract of *Iphionia grantioides* (WMEIG)

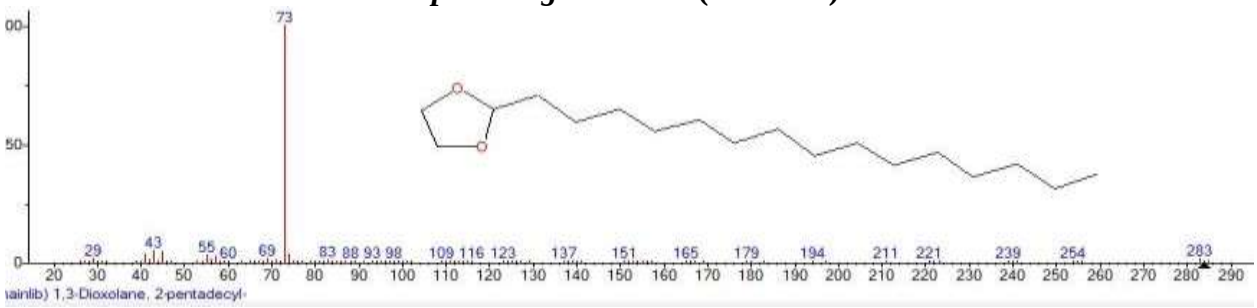


Figure 9: Mass spectra of 1,3-Dioxolane, 2-pentadecyl-of Whole Plant methanol extract of *Iphionia grantioides* (WMEIG)

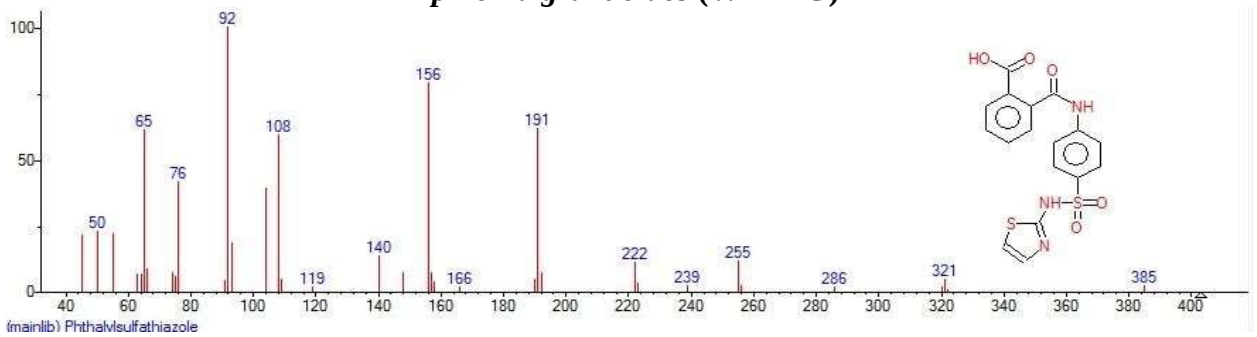


Figure 10: Mass spectra of Phthalylsulfathiazole of Whole Plant methanol extract of *Iphionia grantioides* (WMEIG)

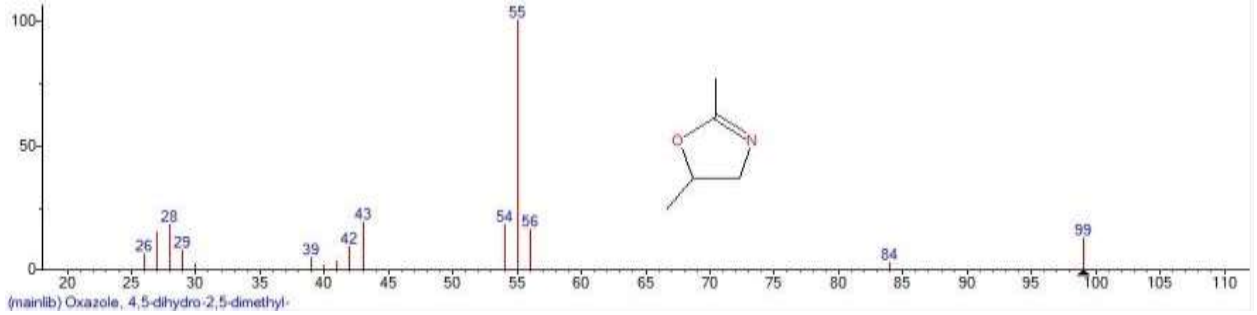


Figure 11: Mass spectra of 0xazole, 4,5-dihydro-2,5-dimethyl of Whole Plant methanol extract of *Iphionia grantioides* (WMEIG)

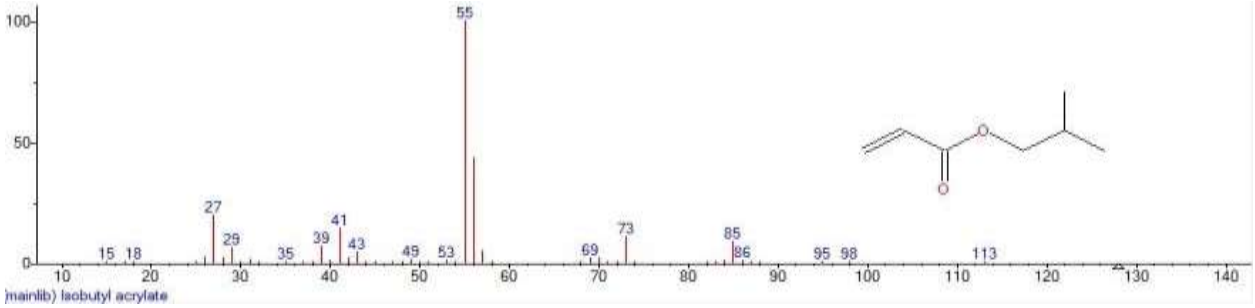


Figure 12: Mass spectra of Isobutyl acrylate of Whole Plant methanol extract of *Iphionia grantioides* (WMEIG)

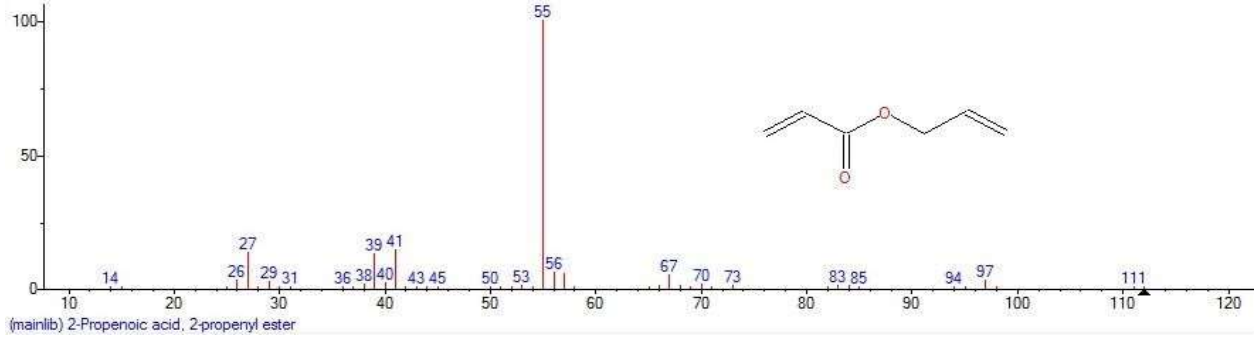


Figure 13: Mass spectra of 2-Propenoic-acid,2-propenyl ester of Whole Plant methanol extract of *Iphionia grantioides* (WMEIG)

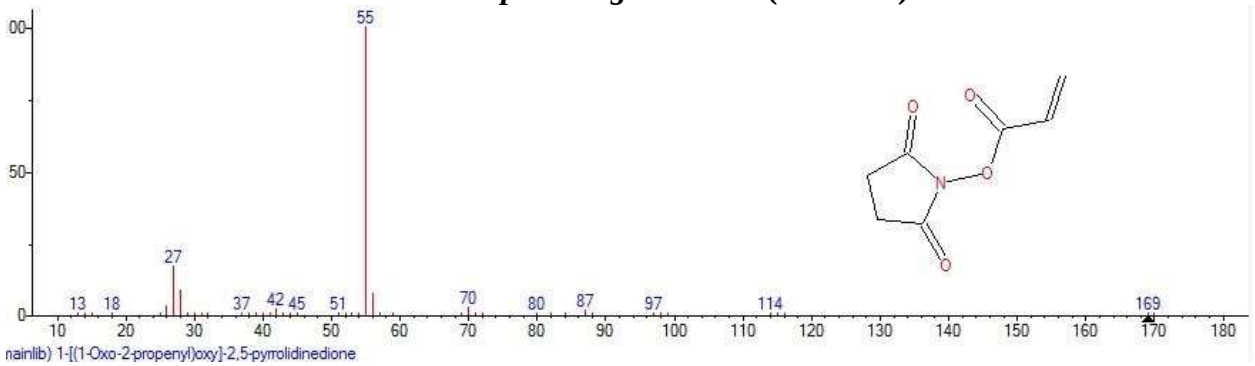


Figure 14: Mass spectra of 1-[(1-Oxo-2-propenyl) oxy}-2,5-pyrrolidinedione of Whole Plant methanol extract of *Iphionia grantioides* (WMEIG)

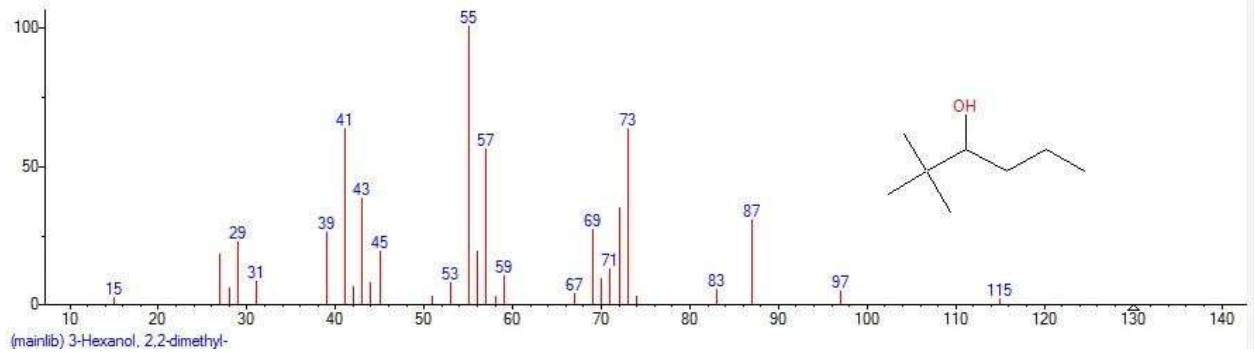


Figure 15: Mass spectra of 3-Hexanol, 2,2-dimethyl of Whole Plant methanol extract of *Iphionia grantioides* (WMEIG)

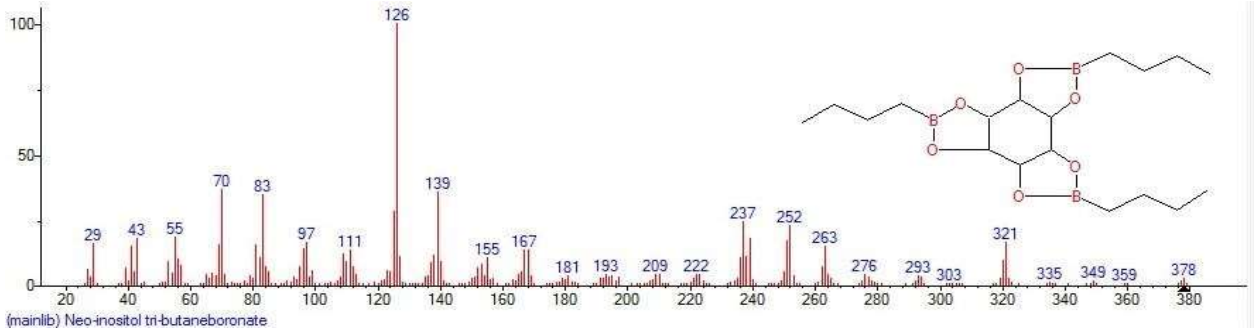


Figure 16: Mass spectra of Neo-inositol tri-butane boronate of Whole Plant methanol extract of *Iphionia grantioides* (WMEIG)

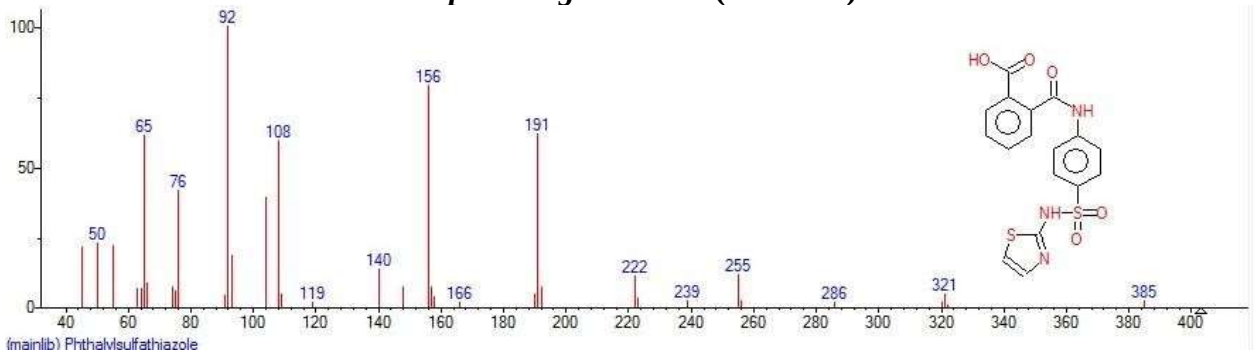


Figure 17: Mass spectra of Phthalylsulfathiazole of Whole Plant methanol extract of *Iphionia grantioides* (WMEIG)

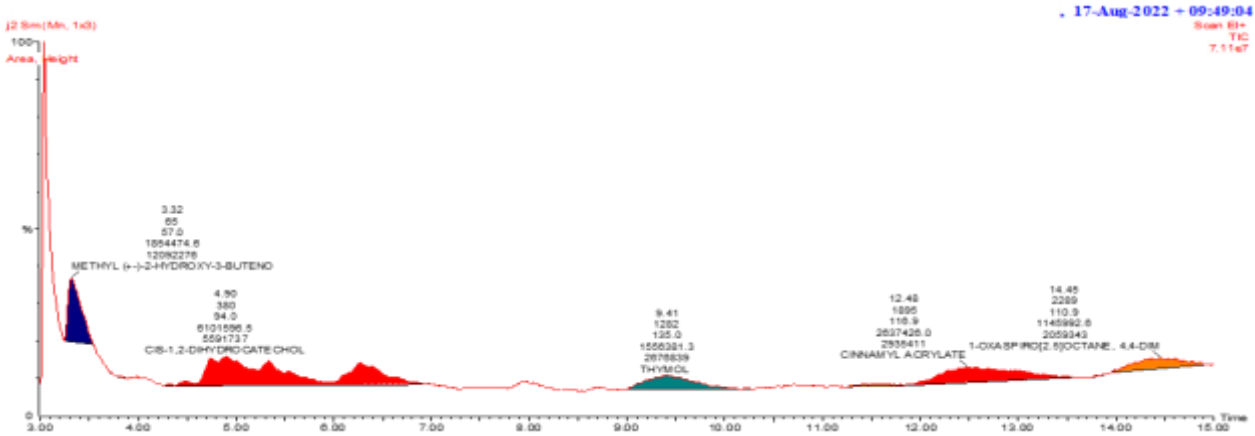


Figure 18: Chromatogram of whole plant n-hexane fraction of *Iphionia grantioides* (WHFIG)

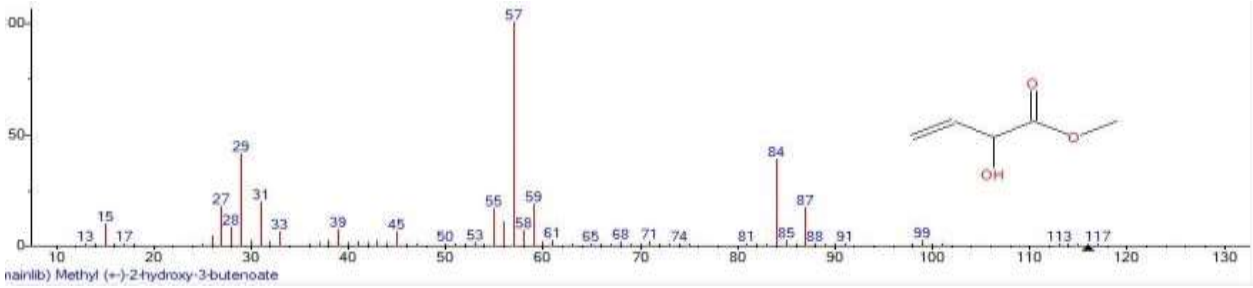


Figure 19: Mass spectra of Methyl (+-)-2- hydroxyl-3-butenoate whole plant n-hexane fraction of *Iphionia grantioides* (WHFIG)

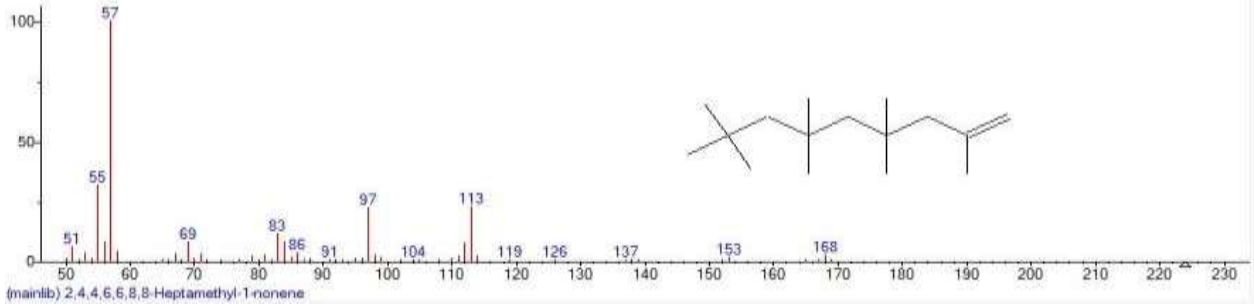


Figure 20: Mass spectra of 2,4,4,6,6,8,8-Heptamethyl -1-nonene whole plant n-hexane fraction of *Iphionia grantioides* (WHFIG)

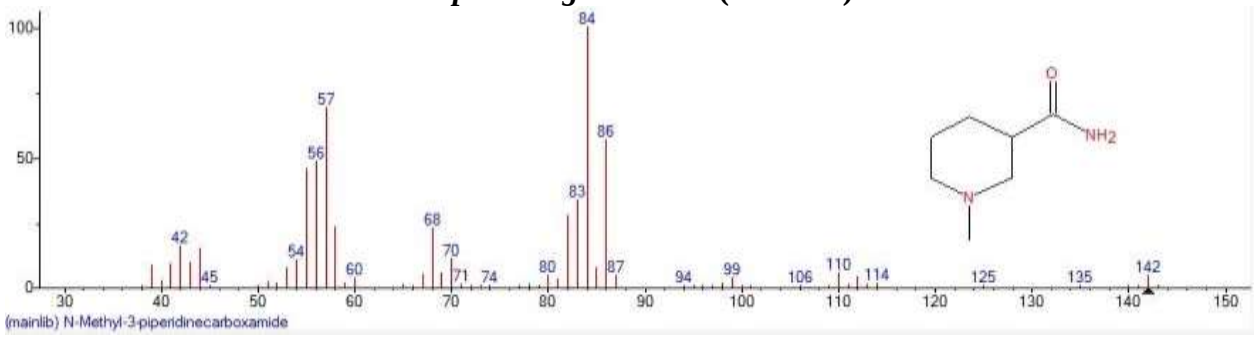


Figure 21: Mass spectra of N-M-methyl-3-piperidinecarboxamide whole plant n-hexane fraction of *Iphionia grantioides* (WHFIG)

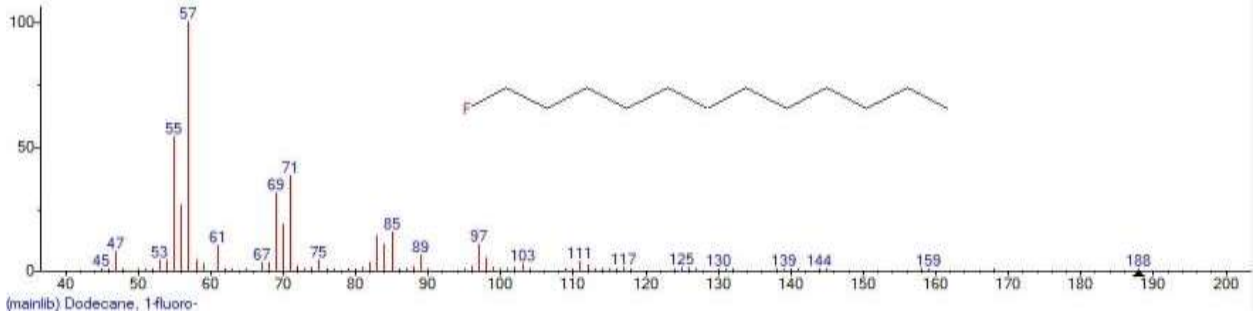


Figure 22: Mass spectra of Dodecane, 1-fluoro whole plant n-hexane fraction of *Iphionia grantioides* (WHFIG)

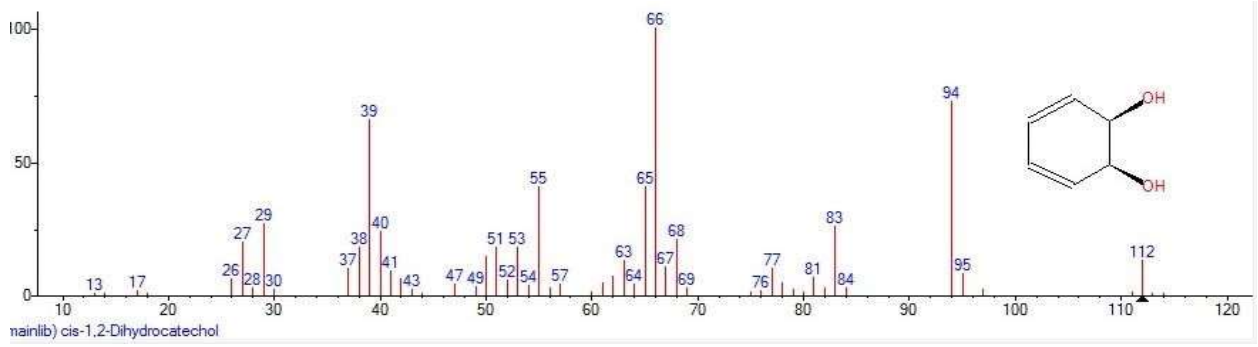


Figure 23: Mass spectra of Cis-1,2-dihydrocatechol whole plant n-hexane fraction of *Iphionia grantioides* (WHFIG)

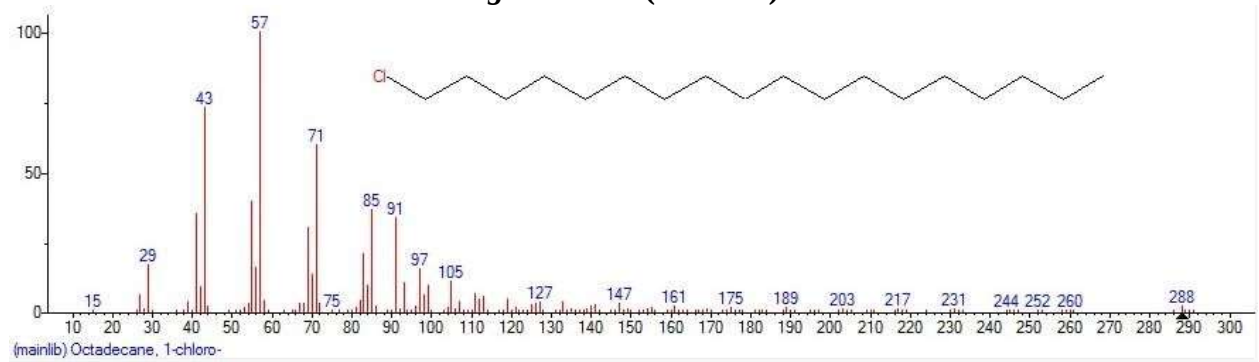


Figure 24: Mass spectra of Octadecane, 1-chloro- whole plant n-hexane fraction of *Iphionia grantioides* (WHFIG)

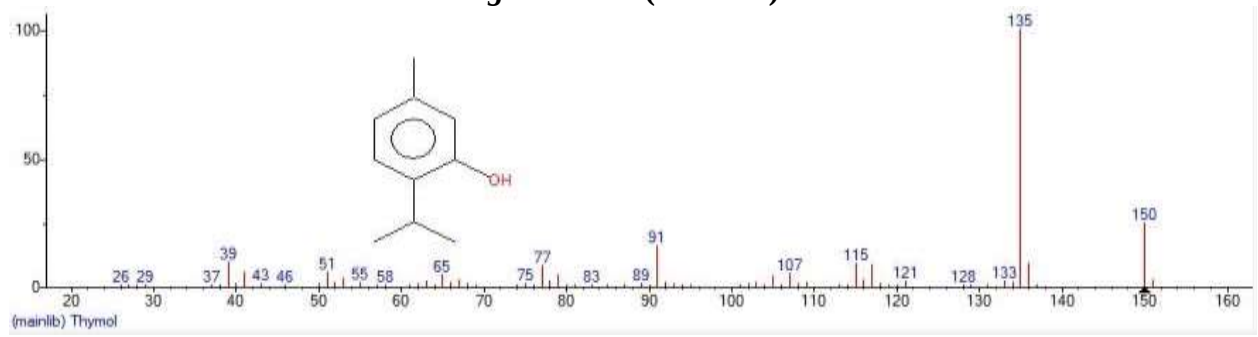


Figure 25: Mass spectra of Thymol- whole plant n-hexane fraction of *Iphionia grantioides* (WHFIG)

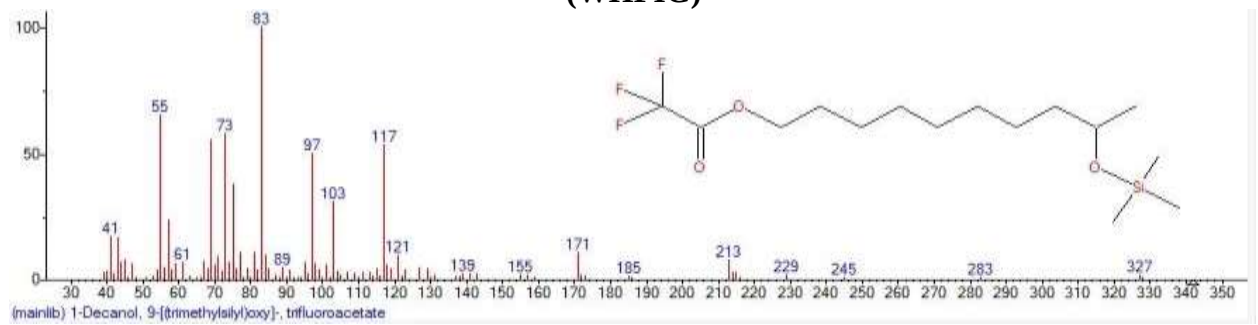


Figure 26: Mass spectra of Cinnamyl acrylate - whole plant n-hexane fraction of *Iphionia grantioides* (WHFIG)

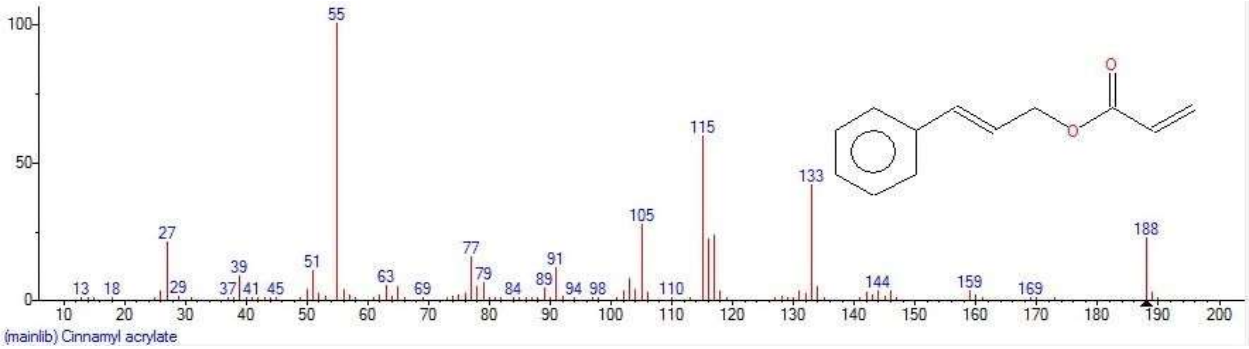


Figure 27: Mass spectra of Cinnamyl acrylate - whole plant n-hexane fraction of *Iphionia grantioides* (WHFIG)

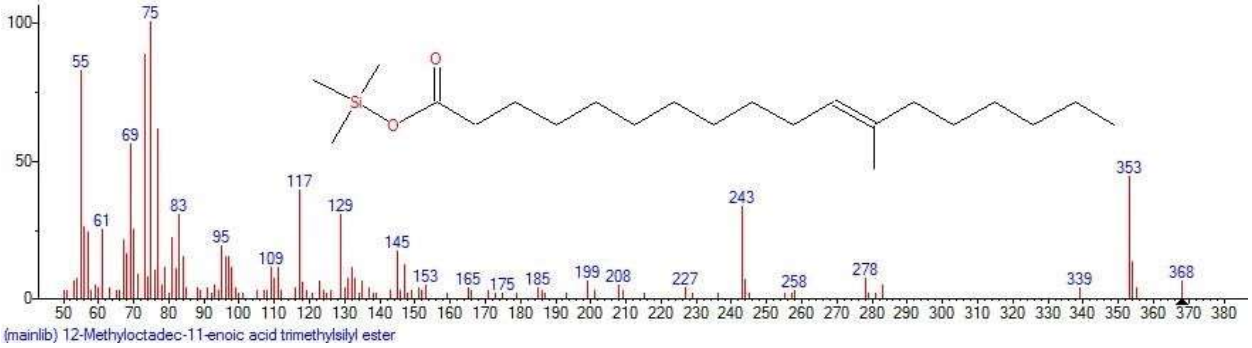


Figure 28: Mass spectra of 12-methyloctadec-11-enoic acid trimethyl silyl ester whole plant n-hexane fraction of *Iphionia grantioides* (WHFIG)

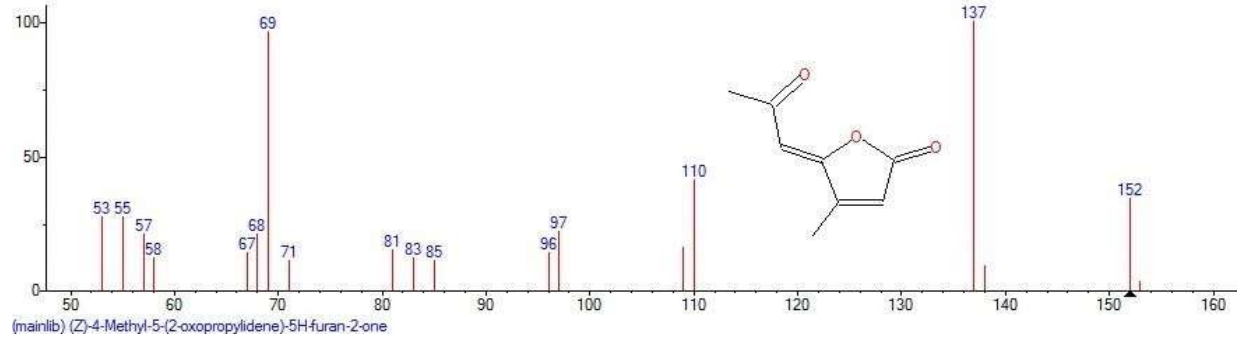


Figure 29: Mass spectra of (Z)-4-methyl-5-(2-oxopropylidene)-5H-Furan-2-one- whole plant n-hexane fraction of *Iphionia grantioides* (WHFIG)

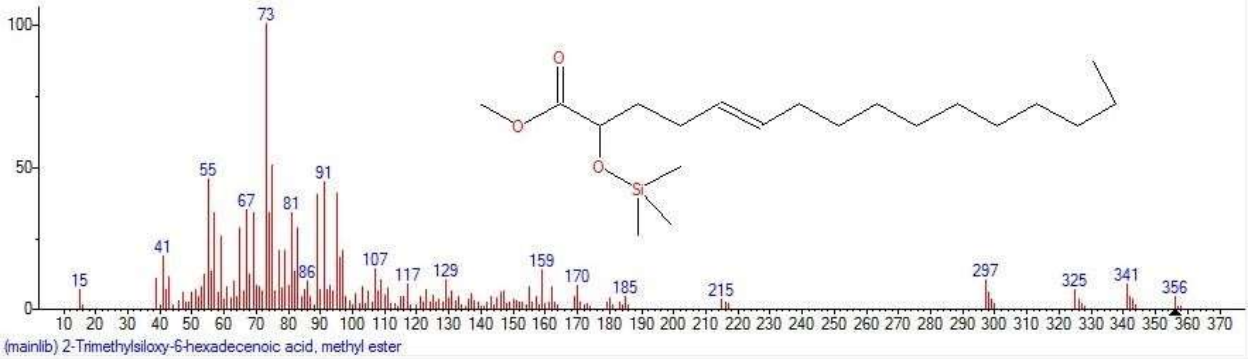


Figure 30: Mass spectra of 2-Trimethylsiloxy-6-Hexadecenoic acid, methyl ester whole plant n-hexane fraction of *Iphionia grantioides* (WHFIG)

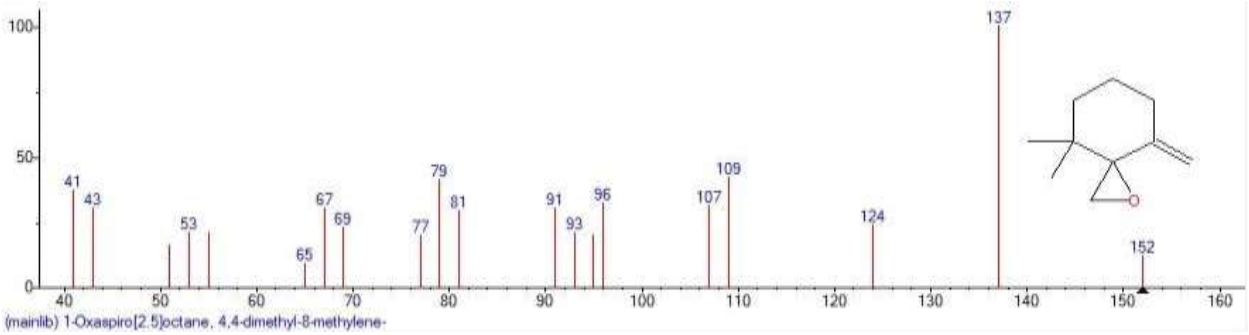


Figure 31: Mass spectra of 1-Oxaspiro {2,5] octane, 4,4-dimethyl-8-methylenewhole whole plant n-hexane fraction of *Iphionia grantioides* (WHFIG)

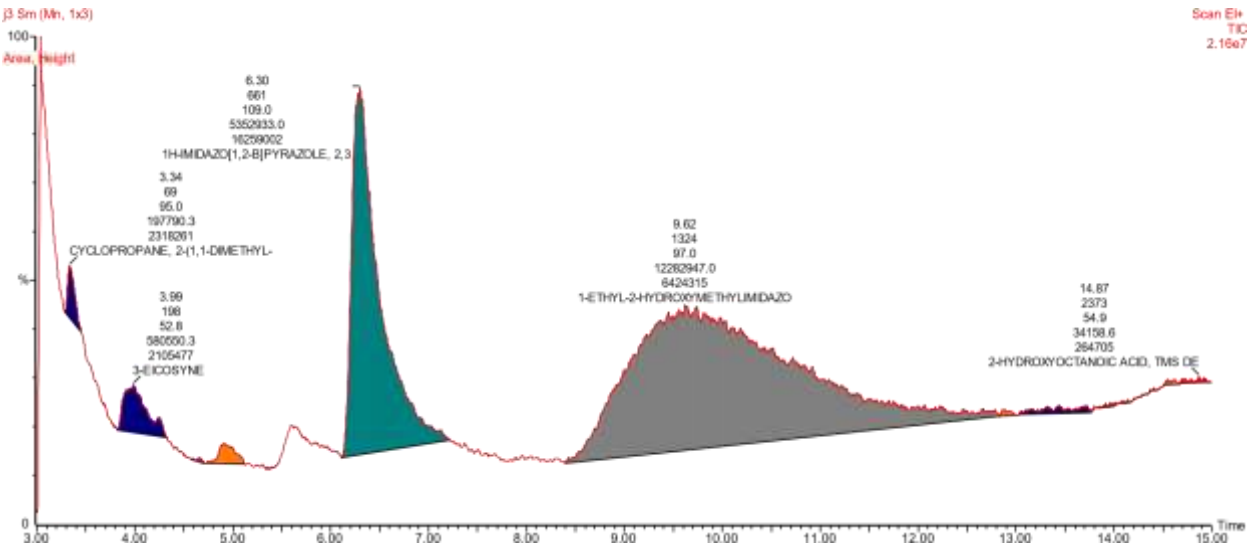


Figure 32: Chromatogram of Whole plant aqueous fraction of *Iphionia grantioides* (WAFIG)

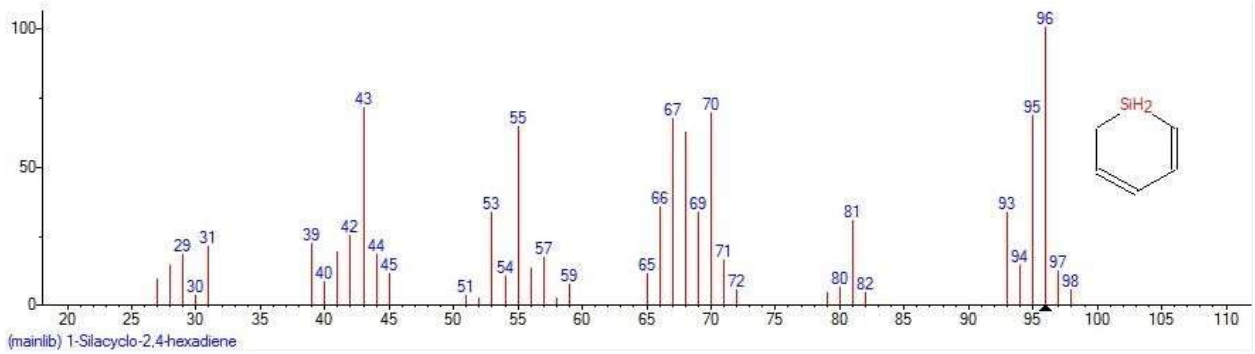


Figure 33: Mass spectra of 1-silacyclo-2,4-hexadiene Whole plant aqueous fraction of *Iphionia grantioides* (WAFIG)

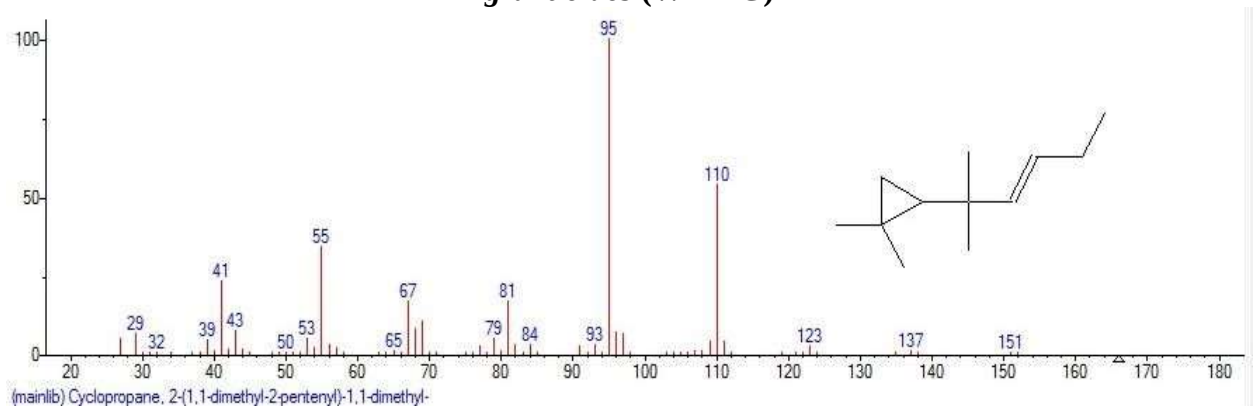


Figure 34: Mass spectra of Cyclopropane, 2-(1,1-dimethyl-2-pentenyl)-1,1-dimethyl Whole plant aqueous fraction of *Iphionia grantioides* (WAFIG)

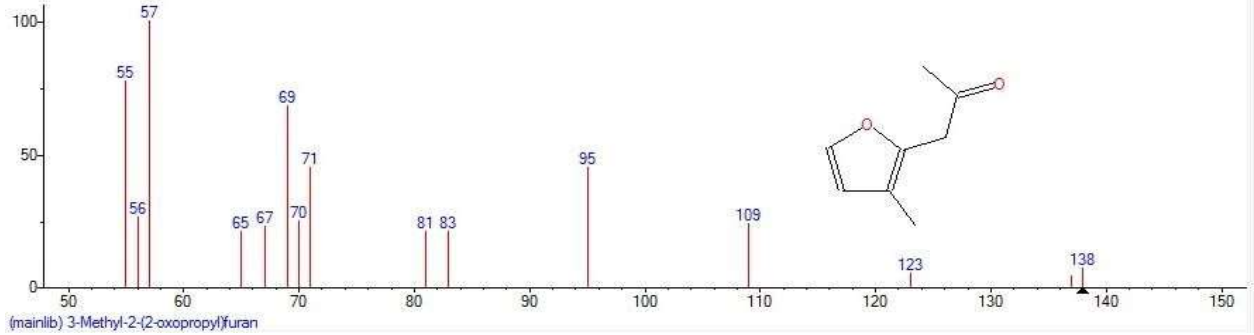


Figure 35: Mass spectra of Cyclopropane, 2-(1,1-dimethyl-2-pentenyl)-1,1-dimethyl Whole plant aqueous fraction of *Iphionia grantioides* (WAFIG)

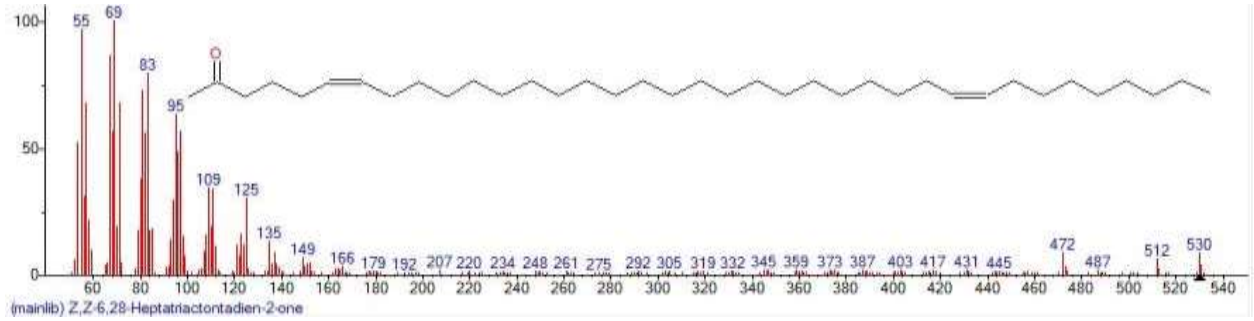


Figure 36: Mass spectra of Z, Z-6,28-Heotatriactontadien-2-one Whole plant aqueous fraction of *Iphionia grantioides* (WAFIG)

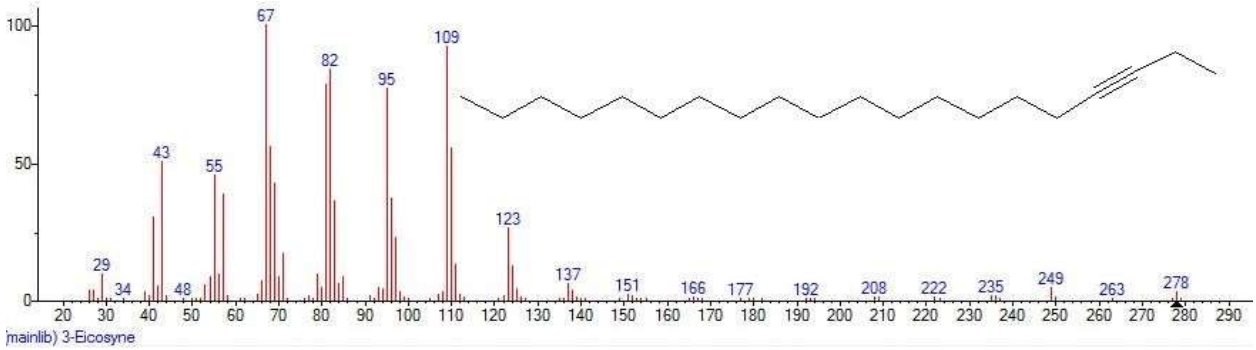


Figure 37: Mass spectra of 3-eicosyne Whole plant aqueous fraction of Iphionia grantioides (WAFIG)

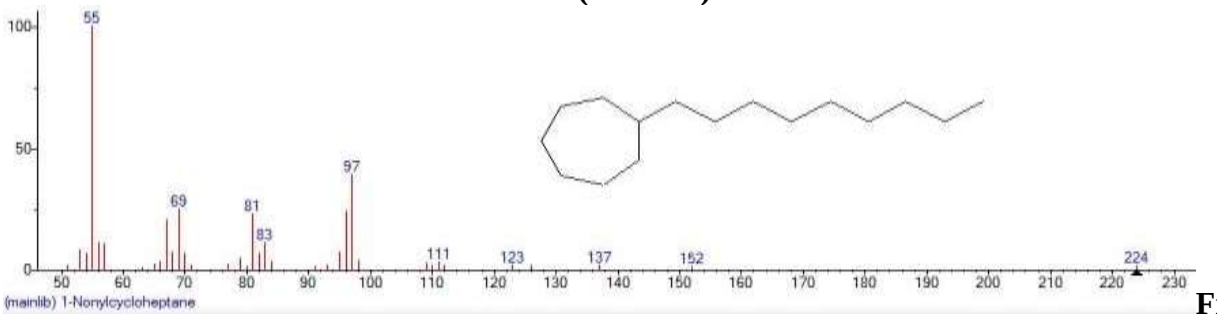


Figure 38: Mass spectra of 1-Nonylcycloheptane Whole plant aqueous fraction of *Iphionia grantioides* (WAFIG)

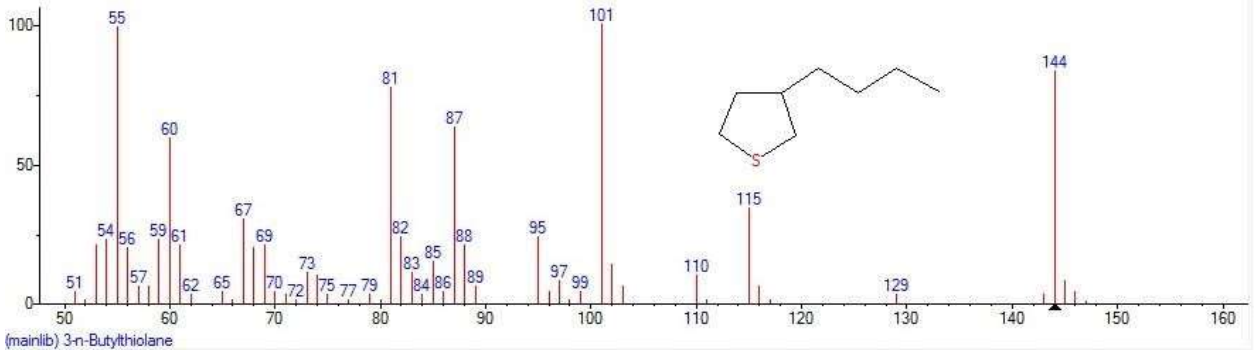


Figure 39: Mass spectra of 3-n-butylthiolane Whole plant aqueous fraction of *Iphionia grantioides* (WAFIG)

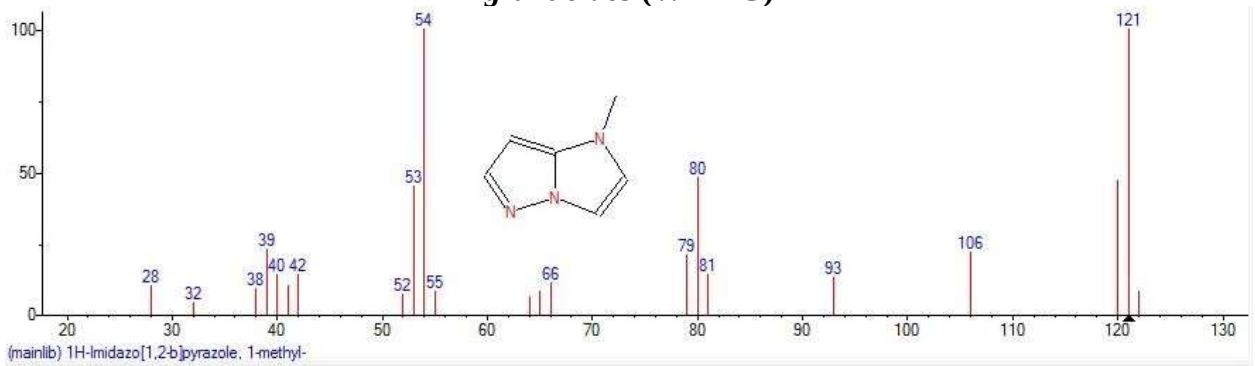


Figure 40: Mass spectra of 1 H-imidazol[1,2-b] pyrazole, 1-methyl- Whole plant aqueous fraction of *Iphionia grantioides* (WAFIG)

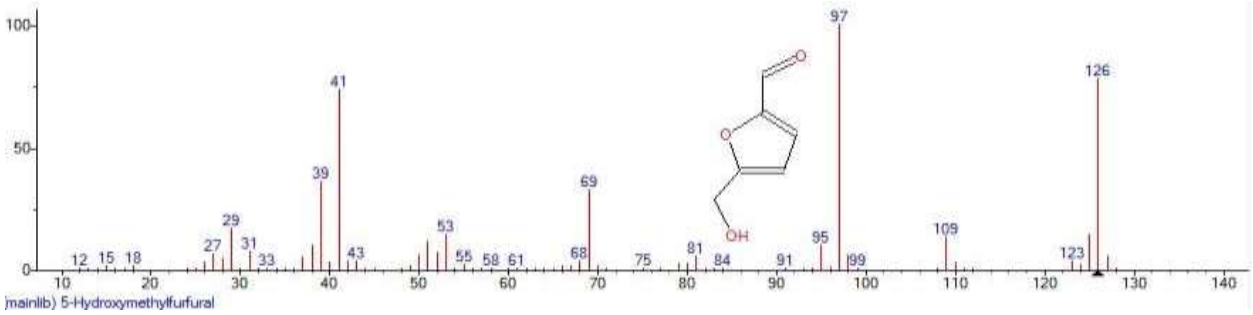


Figure 41: Mass spectra of 5-Hydroxymethylfurfural Whole plant aqueous fraction of *Iphionia grantioides* (WAFIG)

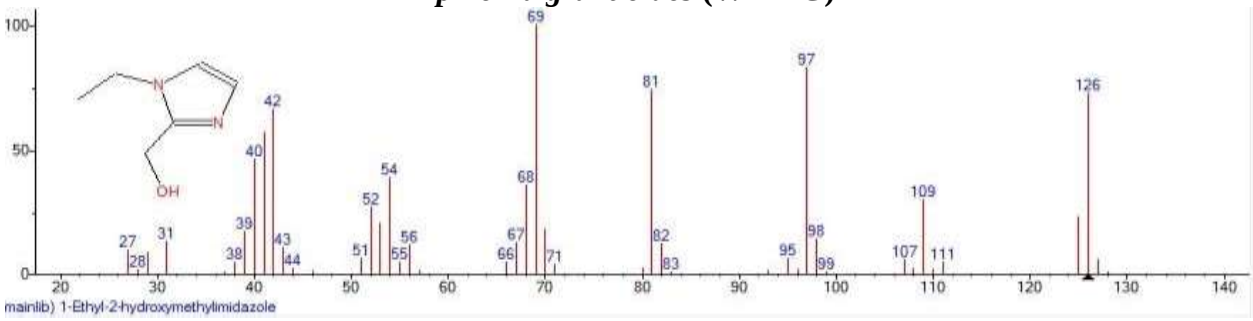


Figure 42: Mass spectra of 1-ethyl-2-hydroxymethylimidazole Whole plant aqueous fraction of *Iphionia grantioides* (WAFIG)

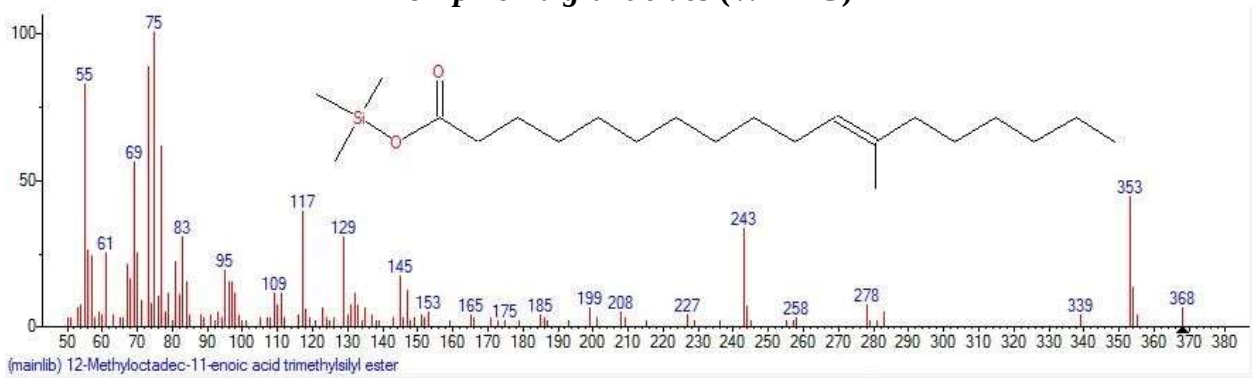


Figure 43: Mass spectra of 12-methyloctadec-11-enoic acid trimethylsilyl ester Whole plant aqueous fraction of *Iphionia grantioides* (WAFIG)

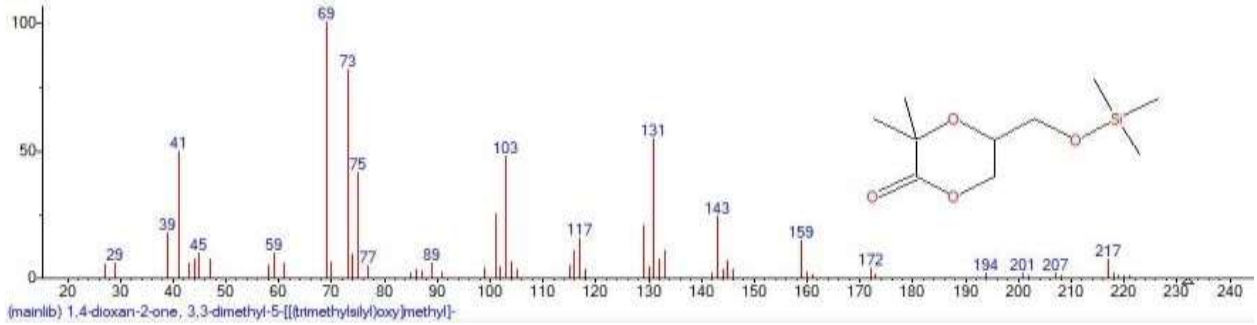


Figure 44: Mass spectra of 1,4-Dioxan-2-one, 3,3-dimethyl-5-[(trimethylsilyl)oxy) methyl] Whole plant aqueous fraction of *Iphionia grantioides* (WAFIG)

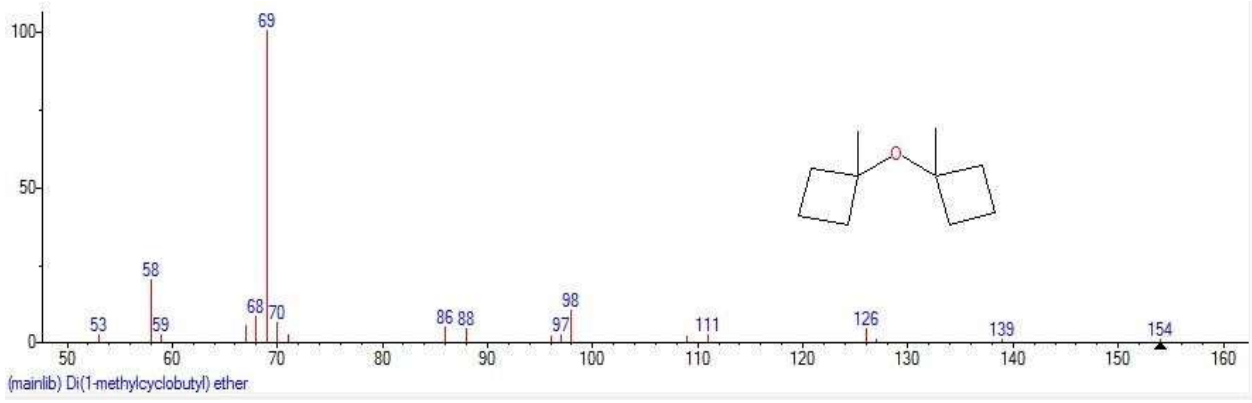


Figure 45: Mass spectra of Di(1-methylcyclobutyl) ether Whole plant aqueous fraction of *Iphionia grantioides* (WAFIG)

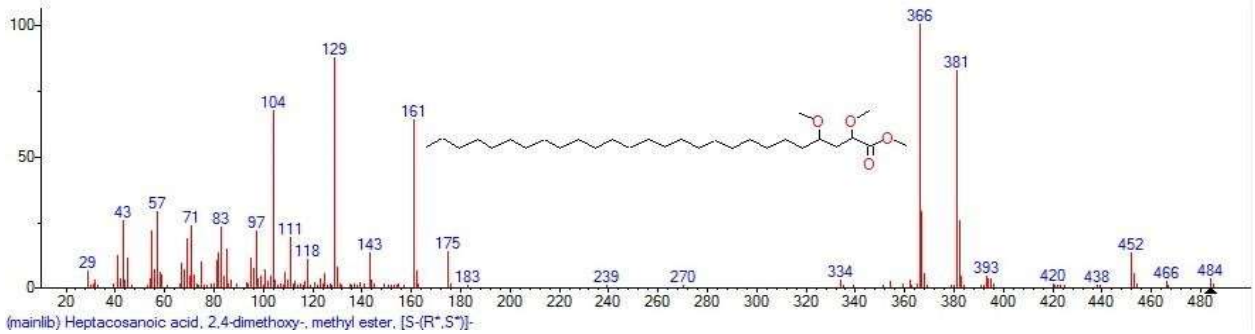


Figure 46: Mass spectra of Heptacosanoic acid, 2,4-dimethoxy-methylester Whole plant aqueous fraction of *Iphionia grantioides* (WAFIG)

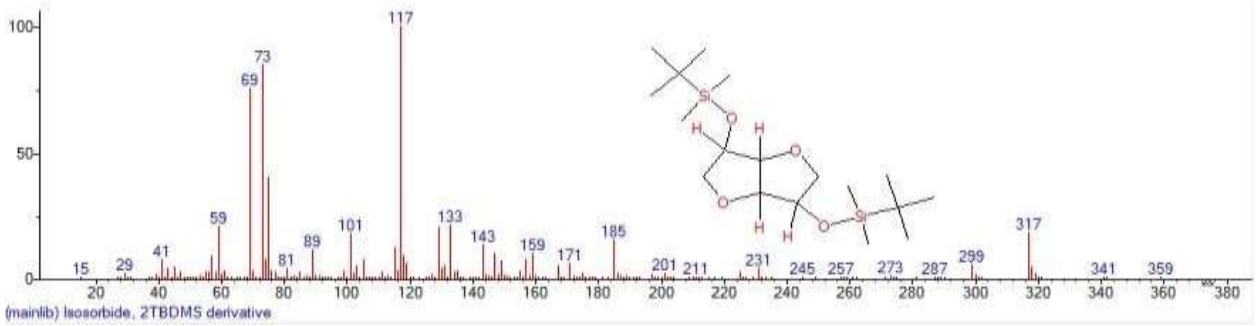


Figure 47: Mass spectra of Isosorbide, 2TBDMS derivative Whole plant aqueous fraction of *Iphionia grantioides* (WAFIG)

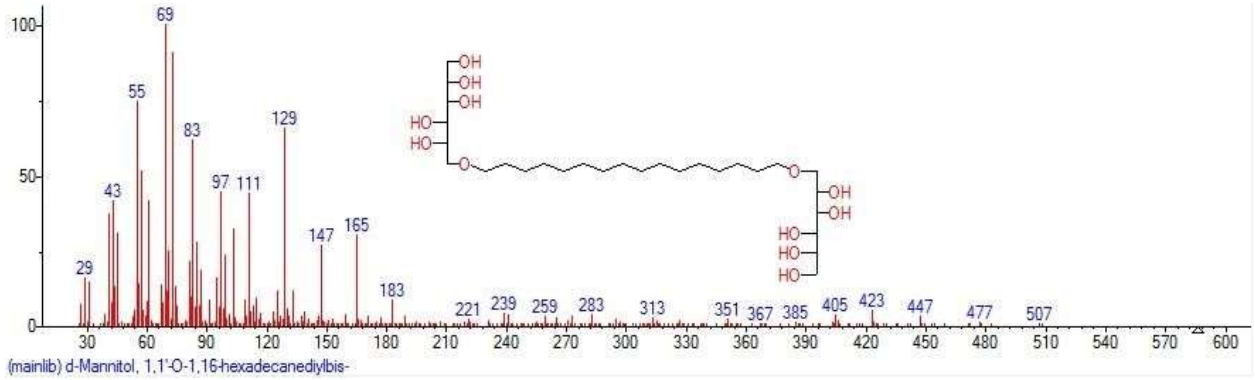


Figure 48: Mass spectra of d-mannitol, 1,1-o-1,16-hexadecanediylbis-Whole plant aqueous fraction of *Iphionia grantioides* (WAFIG)

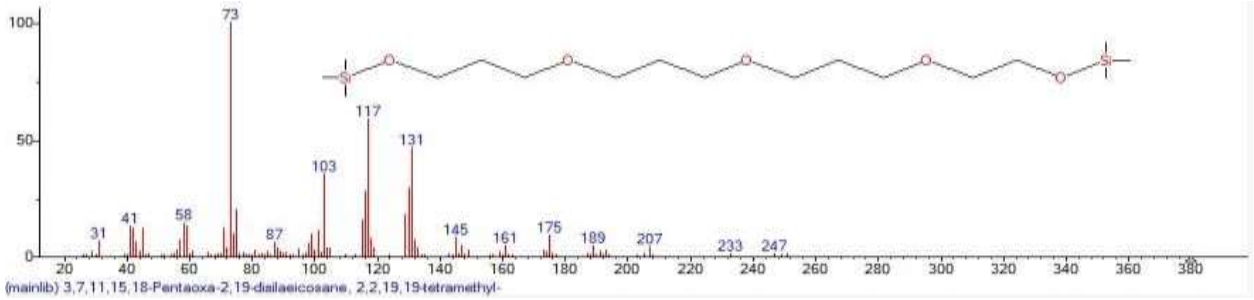


Figure 49: Mass spectra of 3,7,11,15,18-pentaoxa-2,19-disilaeicosane,2,2,19,19tetramethyl - Whole plant aqueous fraction of *Iphionia grantioides* (WAFIG)

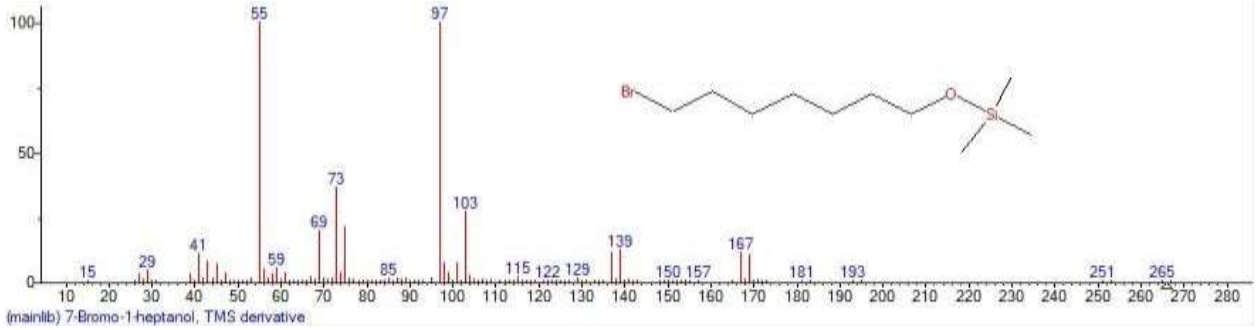


Figure 50: Mass spectra of 7-bromo-1-heptanol. TMS derivative Whole plant aqueous fraction of *Iphionia grantioides* (WAFIG)

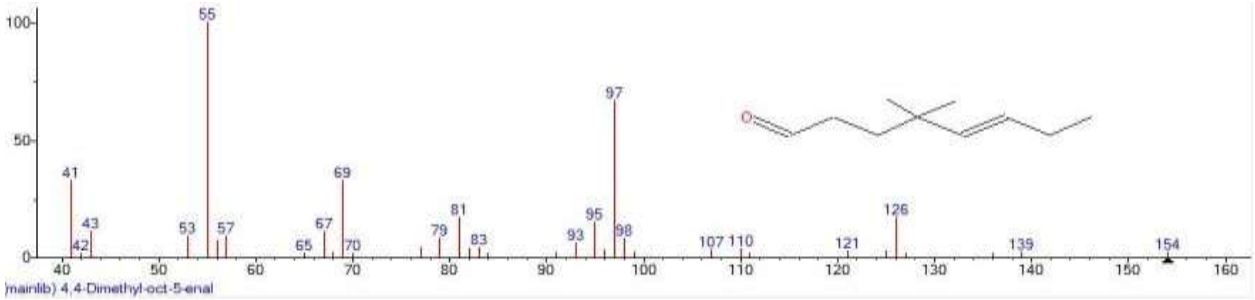


Figure 51: Mass spectra of 4,4-Dimethyl-oct-5-enal Whole plant aqueous fraction of *Iphionia grantioides* (WAFIG)

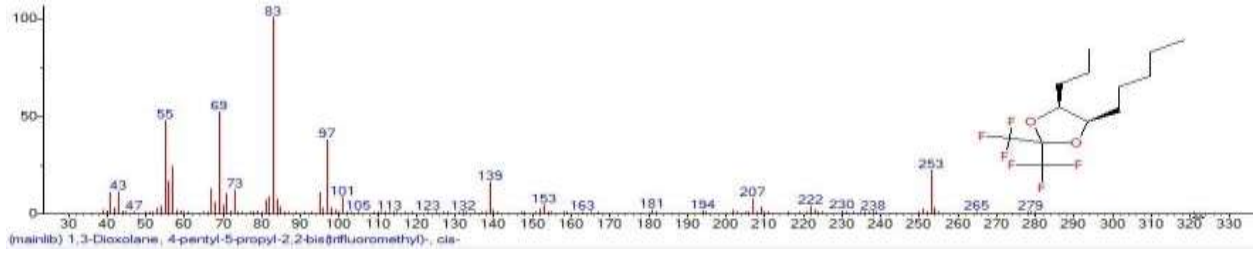


Figure 52: Mass spectra of 1,3-dioxolane, 4-pentyl-5-propyl-2,2-bis(trifluoromethyl)-CisWhole plant aqueous fraction of *Iphionia grantioides* (WAFIG)

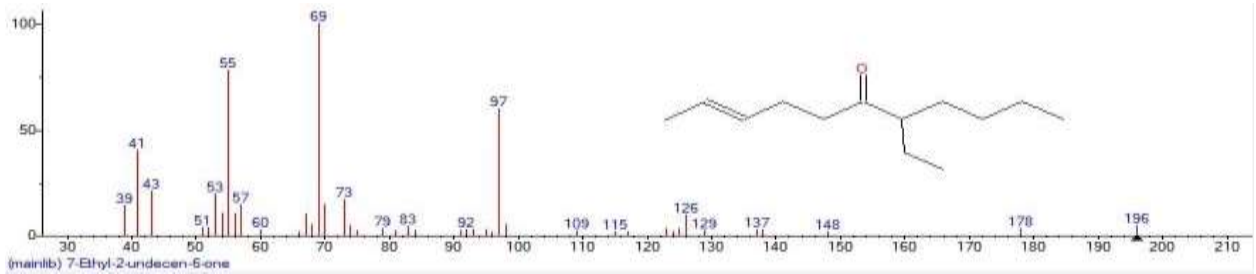


Figure 53: Mass spectra of 7-ethyl-2-undecen-6-one Whole plant aqueous fraction of *Iphionia grantioides* (WAFIG)

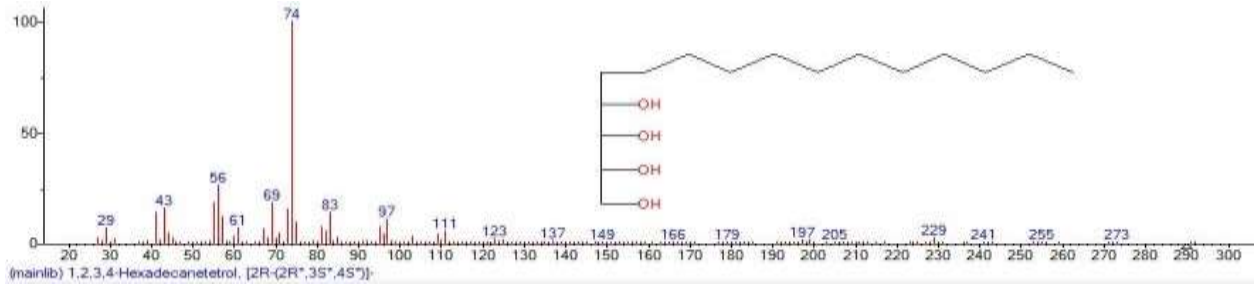


Figure 54: Mass spectra of 1,2,3,4-Hexadecanetetrol Whole plant aqueous fraction of *Iphionia grantioides* (WAFIG)

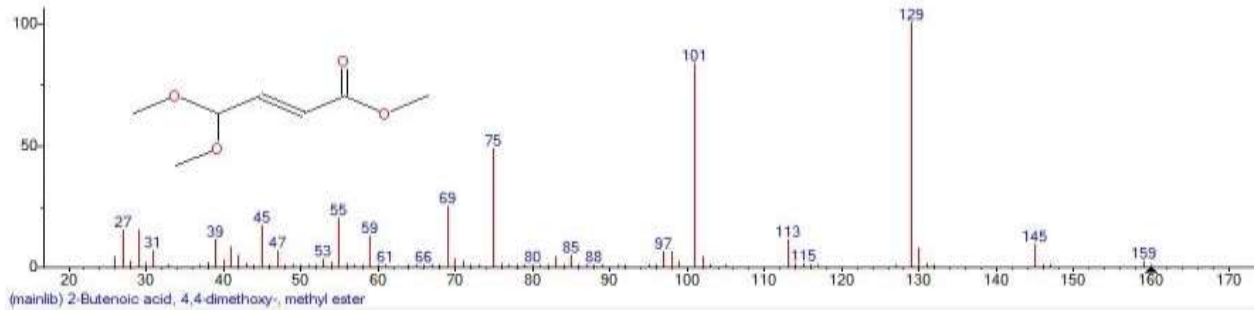


Figure 55: Mass spectra of 2-Butenoic acid, 4,4-dimethoxy- methyl ester Whole plant aqueous fraction of *Iphionia grantioides* (WAFIG)

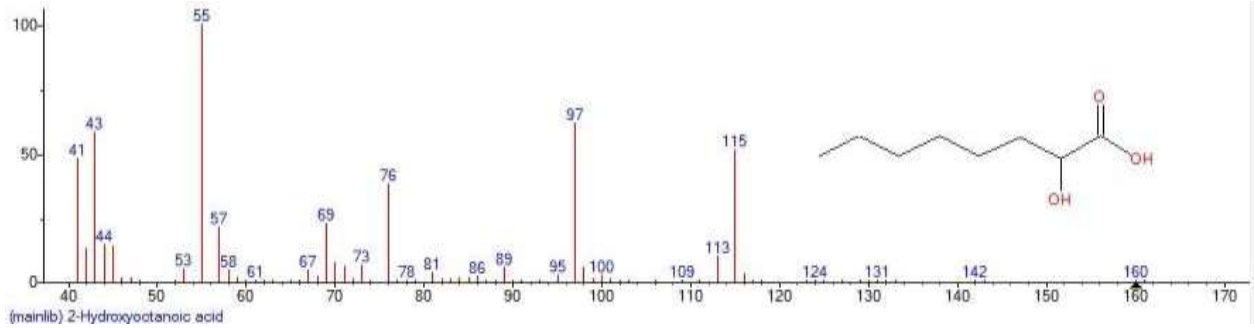


Figure 56: Mass spectra of 2-hydroxyoctanoic acid Whole plant aqueous fraction of *Iphionia grantioides* (WAFIG)

3- Conclusion

Plants being the main source of different medicine, plays an essential and significant role in the health of both plants as well as human. One extract, for instance, whole plant methanol extract of *Iphionia grantioides* (WMEIG), and two fractions, such as whole plant n-hexane fraction of *Iphionia grantioides* (WHFIG), whole plant aqueous fraction of *Iphionia grantioides* (WAFIG) extracted and fractionated from the medicinal plant of Baluchistan, *Iphionia grantioides*. None of the extract and fractions of *Iphionia grantioides* showed antileishmanial activities and having IC_{50} above 100. The standard drug used for antileishmanial activities is amphoterinin B and pentamidin with IC_{50} value 3.41 ± 0.02 and 4.56 ± 0.01.

The extract and fractions of *Iphionia grantioides* were inactive against bacterial strains. Ofloxacin was the standard drug used against bacterial strains with percent inhibition 92.54%, 92.41%, 93.05%, 92.68% and 92.37% against *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli* and *Bacillus subtilis*. All extracts and fractions of *Iphionia grantioides* exhibited no antifungal activities. Miconazole and amphotericin B were the standard drugs used against seven strains of fungi for instanc *Aspergillus niger*, *Microsporum canis*, *Fusarium lini*, *Candida glabarata, Aspergillus fumigatus*, *Candida albicans* and *Trichphyton rubrum*.

None of the extract and fractions of *Iphionia grantioides* showed anticancer activities against HeLa cell line. The standard drug, Doxorubicin inhibited the growth of HeLa cell line 100% with IC_{50} 0.9± 0.14. None of the extract and fractions of *Iphionia grantioides* showed anticancer activities against PC3 cell line. The standard drug, Doxorubicin inhibited the growth of HeLa cell line 89.9% with IC_{50} 1.9± 0.14. None of the extract and fractions of *Iphionia grantioides* showed anticancer activities against 3T3 cell line. The standard drug Doxorubicin inhibited 96.2% the growth of the cell line with IC_{50} value 0.1± 0.02.

None of the extract and fractions of *Iphionia grantioides* showed anti-inflammatory activities. The standard drug used for anti-inflammatory activity is Ibuprofen with 73.2% inhibition and IC_{50} 11.2± 1.4 ug/mL. None of the extract and fractions of *Iphionia grantioides* showed lethality. The standard drug used for Brine Shrimp Lethality Assay is Ibuprofen with IC_{50}11.2± 1.4 ug/mL.

GC-MS studies revealed that whole plant methanol extract of *Iphionia grantioides* (WMEIG) consists of 16 compounds, for instance, Dodecane, 1-fluoro, 3-Methyl-2-(2-oxopropyl) furan, Heptane, 1,1-Oxybis-, Arabino-Hex-1-Enitol. 1,5-Anhydro-2-deoxy, 2-propenoic acid, Butyl ester, 2-Undpcene, 6-methyl, 2,4,4-Trimethyal-1-pentanol, 1,3-Dioxolane, 2-pentadecyl-, Sydnone, 3,3-tetramethylenedi-, 0xazole, 4,5-dihydro-2,5-dimethyl-, Isobutyl acrylate, 2Propenoic-acid,2-propenyl ester, 1-[(1-Oxo-2-propenyl) oxy}-2,5-pyrrolidinedione, 3-Hexanol, 2,2-dimethyl, Neo-inositol tri-butane boronate, Phthalylsulfathiazole.

Whole plant n-hexane fraction of *Iphionia grantioides* (WHFIG) consists of 13 compounds for instance, Methyl(+-)-2- hydroxyl-3-butenoate, 2,4,4,6,6,8,8-Heptamethyl -1-nonene, N-Mmethyl-3-piperidinecarboxamide, Dodecane, 1-fluoro, Cis-1,2-dihydrocatechol, Octadecane, 1chloro- , Thymol, 1-Decanol, 9-[(trimethyl silyl)oxy]-trifluoroacetate, Cinnamyl acrylate, 12methyloctadec-11-enoic acid trimethyl silyl ester, (Z)-4-methyl-5-(2-oxopropylidene)-5H-Furan2-one,2-Trimethylsiloxy-6-Hexadecenoic acid, methyl ester, 1-Oxaspiro{2,5] octane, 4,4dimethyl-8-methylene.

Whole plant aqueous fraction of *Iphionia grantioides* (WAFIG) consists of 25 compounds for instance, 1-silacyclo-2,4-hexadiene, Cyclopropane, 2-(1,1-dimethyl-2-pentenyl)-1,1-dimethyl, 3 methyl-2-(2-oxopropyl) furan, Z, Z-6,28-Heotatriactontadien-2-one, 3-eicosyne, 1Nonylcyclo heptane, 3-n-butylthiolane, 1 H-imidazole[1,2-b] pyrazole, 1-methyl-, 5Hydroxymethy lfurfural, 1-ethyl-2-hydroxymethylimidazole, 12-methyloctadec-11-enoic acid trimethylsilyl ester, 1,4-Dioxan-2-one, 3,3-dimethyl-5-[(trimethylsilyl)oxy) methyl], Di(1methylcyclobutyl)eter, Heptacosanoic acid, 2,4-dimethoxy-methylester, Isosorbide, 2TBDMS derivative, d-mannitol, 1,1-o-1,16-hexadecanediylbis-,3,7,11,15,18-pentaoxa-2,19- , 7-bromo-1heptanol. TMS derivative, 4,4-Dimethyl-oct-5-enal, 1,3-dioxolane, 4-pentyl-5-propyl-2,2bis(trifluoromethyl)-cis-, 7-ethyl-2-undecen-6-one, 1,2,3,4-Hexadecanetetrol, 2-Butenoic acid, 4,4-dimethoxy- methyl ester, 2-hydroxyoctanoic acid, d-mannitol, 1-O-(22-Hydroxydocosyl).

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Conflicts of interest-There are no conflicts of interest

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