

Original Research Article

Screening of Secondary Metabolites Produced in Camel Thorn (*Alhagi maurorum*) Leaves Under Combined Environmental Stresses Using GC-Mass Spectrometry

Rawad Khalaf Hameed^{1*}

¹Department of Biology, College of Science, Tikrit University, Salah Al-Din-Iraq

***Corresponding Author:** Rawad Khalaf Hameed

Department of Biology, College of Science, Tikrit University, Salah Al-Din-Iraq

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Abstract: Plants exhibit physiological, morphological, biochemical and molecular adaptations to maintain growth, development and survival under climate change fluctuations. This research aimed to analyse the secondary metabolites produced in *Alhagi maurorum* leaves under different environmental stresses. Secondary metabolites were extracted using Ethanol solvent using Soxhlet apparatus. FTIR and GC-MS analyses were applied to detect the metabolites produced in Alhagi leaves. The results exhibit the detection of 24 compounds at different peaks that were produced in the camel thorn that was naturally grown in the field under climatic stressors. These compounds are involved in the metabolic and physiological processes to grow and survive. The research concluded that environmental fluctuations have a direct influence on the metabolic compounds produced in the plant to cope with stressors and differ from the results concluded in the literature. The number and type of compounds detected showed that the leaves exhibit morphological adaptations to cope environment rather than metabolic adaptations compared to other plant parts and compared to the literature.

Keywords: Camelthorn, *Alhagi Maurorum*, GC-Mass, Secondary Metabolites SM, Abiotic Stressors.

INTRODUCTION

Plants employ physiological, morphological and metabolic adaptations to cope the environmental changes such as scarce rainfall, high light intensity and extreme temperatures, these stresses impose adverse effects and limiting plant growth, development and survival in extreme environmental fluctuations (Al Kaabi *et al.*, 2023; Faiq & Noori, 2021; Raza, 2021; Zhang *et al.*, 2022). Plants have inbuilt physiological adaptation encompasses larger roots and stem area, thicker cuticle, reducing leaf area and numerous large stomata (Iqbal *et al.*, 2023; Saleh *et al.*, 2020). Osmoprotectants like glycine betaine and proline are synthesized to maintain cellular osmotic balance under drought and salinity stress (Abasi *et al.*, 2024; Goharrizi *et al.*, 2021; Singh *et al.*, 2024). *Alhagi maurorum* commonly known as camelthorn, belongs to *Fabaceae* family, it is well adapted to grows and thrive in multiple areas in the world such as Europe, Asia and North Africa. It is classified as of the richest medicinal plants, it contains active constituents like flavonoids, fatty acids, vitamins, sterols, and alkaloids which manifest diuretic, anti-viral, anti-bacterial, anti-cancer, antidiarrheal, anti-ulcer, anti-inflammatory, analgesic, antipyretic, anti-depressant and other pharmaceutical properties that alleviate the biotic and abiotic stresses (Hameed *et al.*, 2022; Islam & Qadir, 2024; Tavassoli *et al.*, 2020; Yuan *et al.*, 2022). Metabolic and morphological adaptations enable *Alhagi* to survive and thrive in fluctuated and harsh climatic conditions (Mengqi *et al.*, 2023). Due to insufficient studies and focus on the metabolic role of Alhagi leaves to cope combined climatic factors surrounding Alhagi that grown in different areas and most studies focuses on biological activities of secondary metabolites as medicinal constituents, the aims of the research is to evaluate the secondary metabolites affected by environment that produced in *Alhagi maurorum* leaves under combined environmental stresses using GC-Mass technique.

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MATERIALS AND METHODS

Collecting Plant Samples

The samples of *A. maurorum* leaves were gained from the unfarmed, unirrigated areas that were naturally planted and not surrounded by other plants in Iraq/Salah al-Din governorate at the grid N 34° 42' 38" E 43° 45' 53". The samples were identified at the Botany Lab at Tikrit University-College of Science. The samples washed thoroughly using distilled water and kept at room temperature for drying at the end of August 2024 away from direct sunlight.

Preparation of Plant Sample

A. maurorum leaves were ground into powder, Soxhlet apparatus was used for extraction using absolute ethanol solvent. 20 g of powder was placed in the thimble with 500 ml of solvent for 6 hrs. A muslin cloth was used for filtration and rotary evaporator used to purify the sample from solvent remnants then the extract was powdered and saved for further analyses (Mohammed, 2020).

Gas Chromatography- Mass Spectrometry GC-Mass

To detect the chemical constituents of *A. maurorum* leaves, GC-Mass apparatus (GCMS-QP2010 Ultra) was used. 1 ml was the sample volume that was injected in the GC pipe. TR5-MS capillary standard, non-polar column, length 30 m, width 0.25 mm. GC temperature ranged from 400-2500 °C for 50 min. The reaction time was 36 min and the mobile phase was set at 1.0 ml/min (Bharathi & Anand, 2016).

Fourier Transform Infrared Spectroscopy FTIR

The dried extract of *A. maurorum* leaves was analyzed using FTIR technique. 1 mg of crude extract powder was encapsulated in 10 mg of KBr pellet. FTIR spectroscopy (Shimadzu, Japan) was used, and the scan range was 400 – 4000 cm⁻¹ (Mohammed & Abd-alkadhemand, 2022).

RESULTS AND DISCUSSION

Environmental conditions

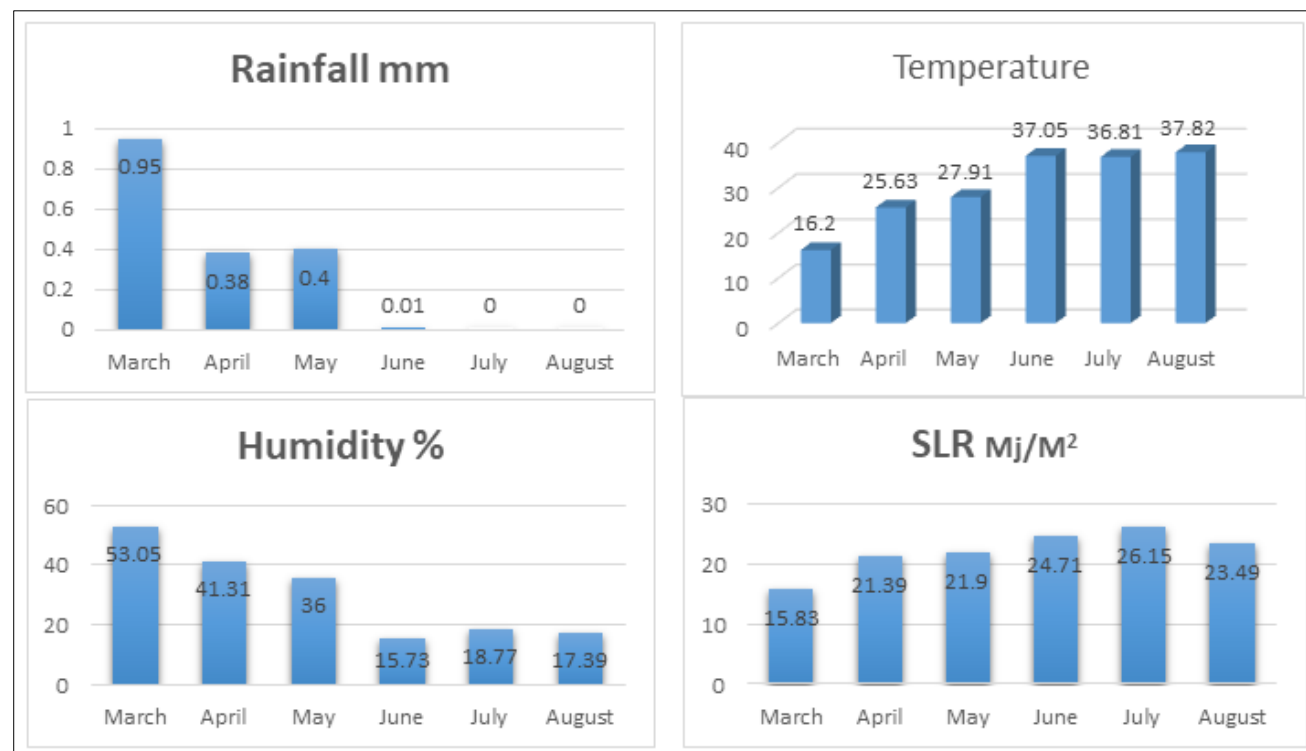


Figure 1: Environmental conditions obtained from the general directorate of environment-Iraqi ministry of environment

FTIR ANALYSIS RESULTS

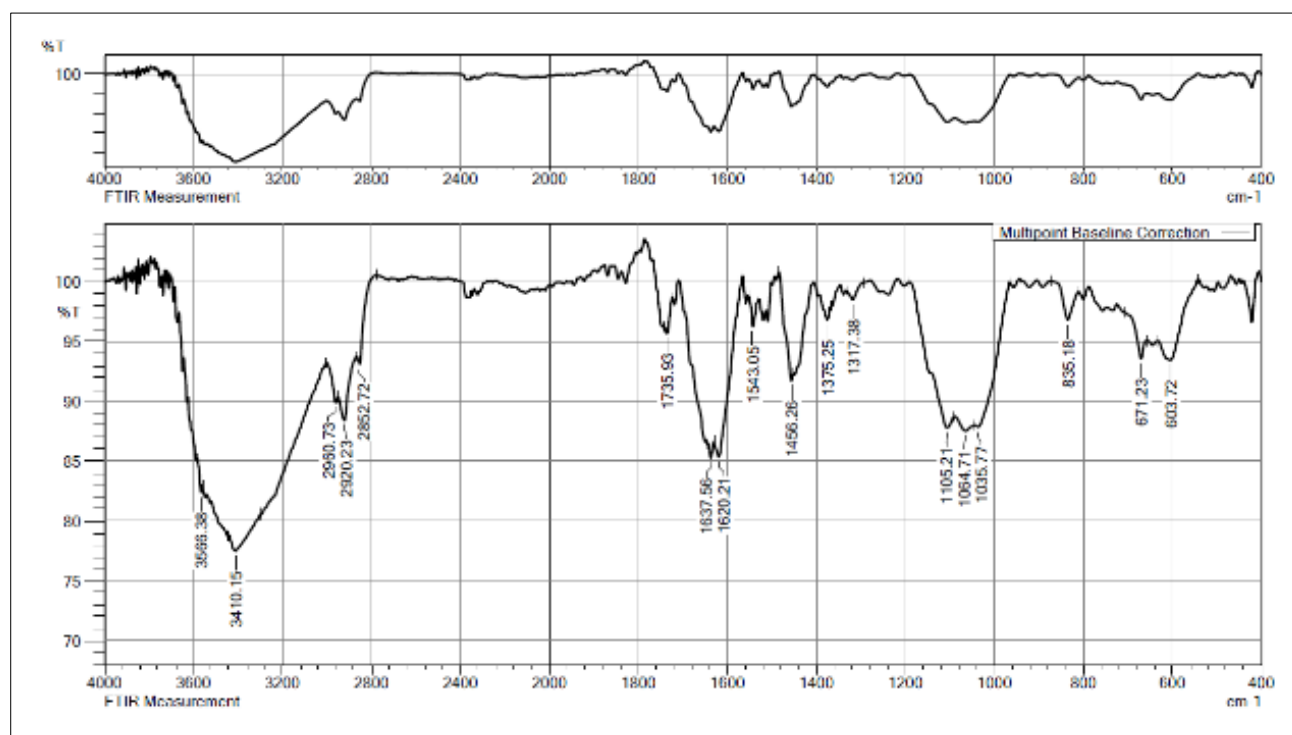


Figure 2: FTIR analysis of plant extract sample

The results of FTIR analysis illustrated in the figure above showed multiple peaks, (603-671 cm^{-1}) indication of the presence of C-Cl chlorinated compounds and C-S sulfides). (835 cm^{-1}) indication of metal-oxygen bonds. (1035, 1064, 1105, 1317, 1375 cm^{-1}) indication of C-O, C-C and indication for alcohols, ethers and esters. (1456 cm^{-1}) indication for C-H bonding. (1543, 1620, 1637, 1735 cm^{-1}) indication of C=C indication for alkenes. (2852, 2920, 2960 cm^{-1}) C-H and indication for hydrocarbon presence. (3410, 3566 cm^{-1}) indication for O-H and the presence of phenols, alcohols, N-H amines and amides.

GC-Mass Analysis

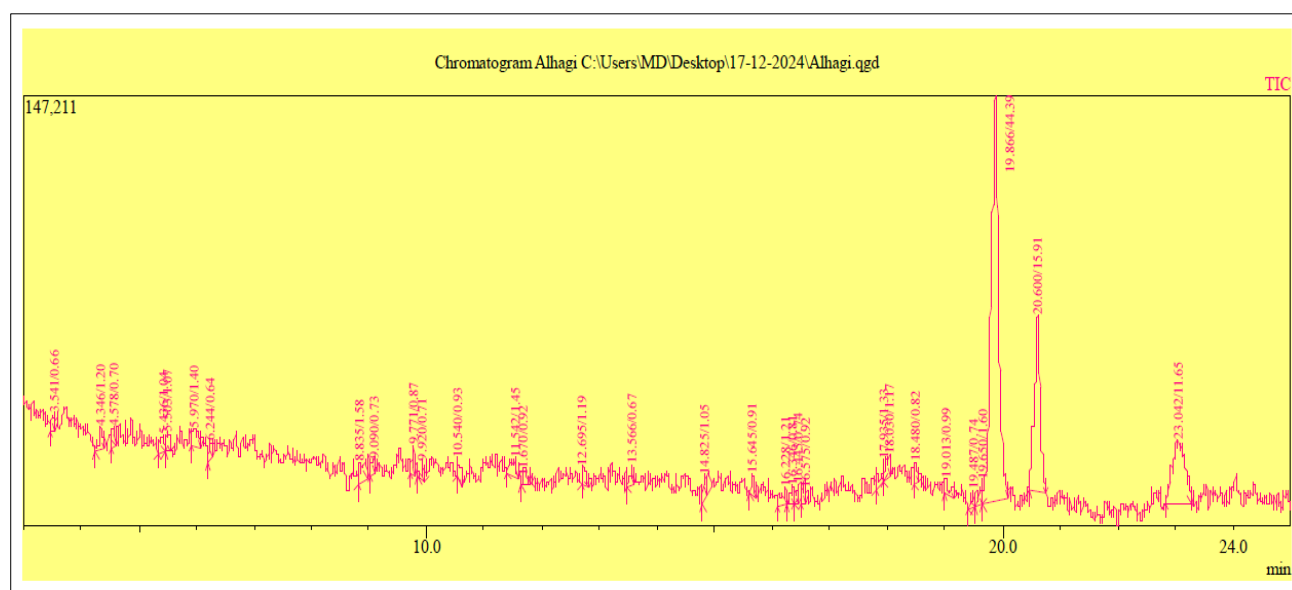
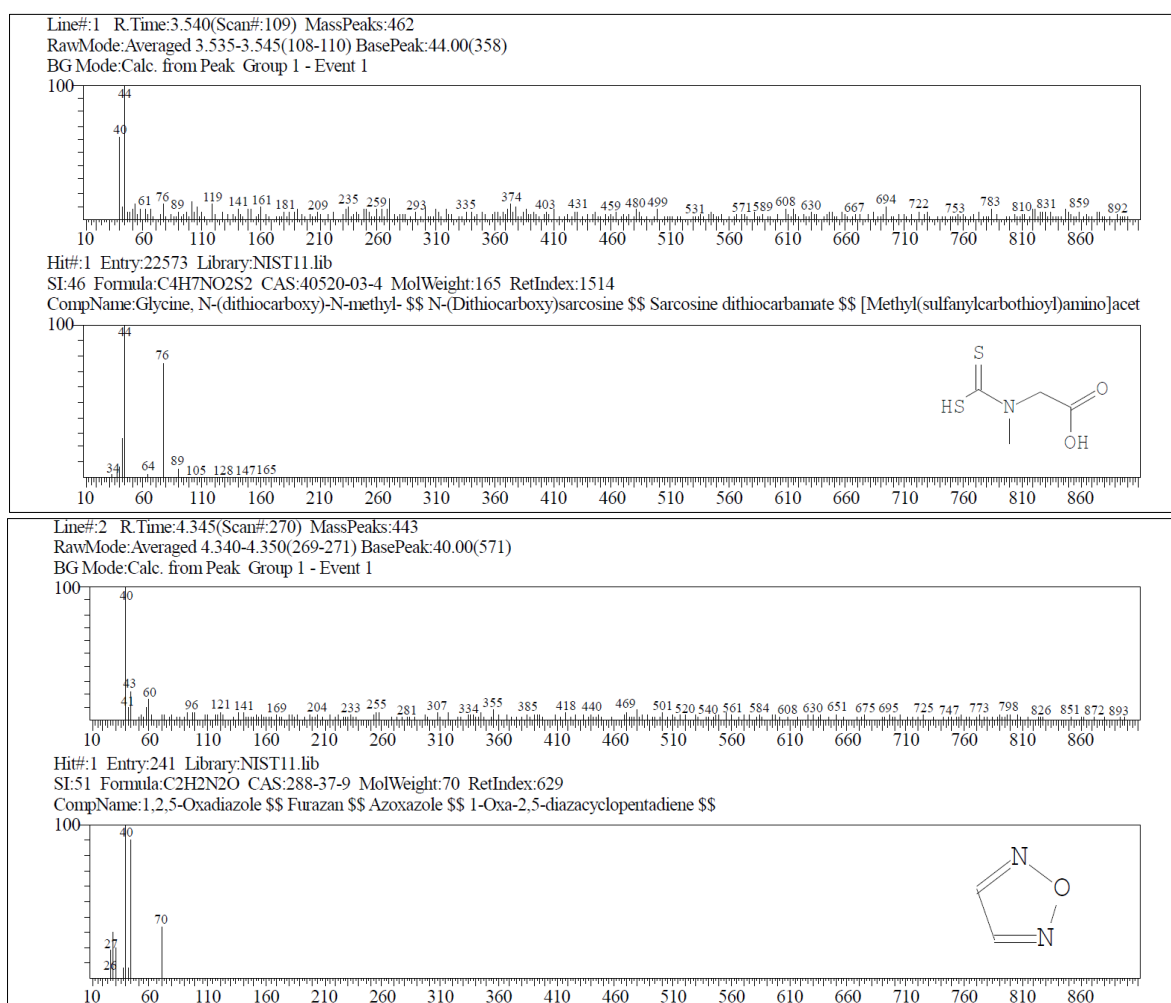


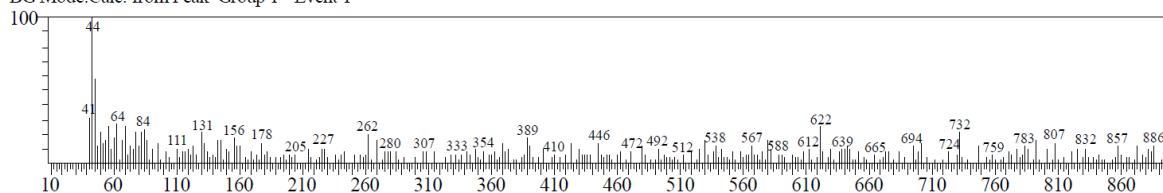
Figure 3: Chromatogram analysis of Alhagi

Peak Report TIC								
Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H Name
1	3.541	3.475	3.555	19213	0.66	6008	1.43	3.20 Glycine, N-(dithiocarboxy)-N-methyl-
2	4.346	4.255	4.410	34975	1.20	8296	1.98	4.22 1,2,5-Oxadiazole
3	4.578	4.535	4.600	20518	0.70	7049	1.68	2.91
4	5.436	5.335	5.455	30364	1.04	6233	1.48	4.87
5	5.503	5.455	5.565	31217	1.07	6869	1.64	4.54 1,1-Cyclopropanedicarboxamide
6	5.970	5.920	6.075	40807	1.40	6254	1.49	6.52 Urea, N,N'-(2-methylpropylidene)bis-
7	6.244	6.205	6.280	18823	0.64	6618	1.58	2.84 3-Butyn-1-ol
8	8.835	8.805	8.965	46114	1.58	7502	1.79	6.15 2H-Pyran, 2-(3-butynloxy)tetrahydro-
9	9.090	9.015	9.105	21390	0.73	4783	1.14	4.47 Pyrazine, 2,6-dimethyl-
10	9.771	9.710	9.795	25431	0.87	10382	2.47	2.45 3-Butynoic acid
11	9.920	9.850	9.975	20765	0.71	6746	1.61	3.08 1,2,3-Butanetriol
12	10.540	10.515	10.615	27217	0.93	7208	1.72	3.78 3-Isothiazolecarboxamide, 4-amino-
13	11.542	11.405	11.590	42231	1.45	6547	1.56	6.45
14	11.670	11.650	11.815	26998	0.92	3162	0.75	8.54 3-(3-Aminopropyl)-4-carboxyfurazan
15	12.695	12.680	12.810	34719	1.19	6375	1.52	5.45 Heptanal, dimethylhydrazone
16	13.566	13.490	13.595	19419	0.67	7086	1.69	2.74 Carbamimidoylsulfanylacetic acid
17	14.825	14.790	14.875	30568	1.05	9144	2.18	3.34 1,3-Oxathiane, 2-ethyl-2-methyl-
18	15.645	15.615	15.720	26655	0.91	7760	1.85	3.43
19	16.228	16.095	16.255	35384	1.21	6455	1.54	5.48 2-Formylhistamine
20	16.346	16.255	16.380	23633	0.81	6766	1.61	3.49 1-Butanol, 4-mercapto-
21	16.445	16.380	16.460	21469	0.74	7088	1.69	3.03 Trifluoromethanesulfonyl imidazole
22	16.575	16.500	16.585	26824	0.92	5165	1.23	5.19
23	17.935	17.790	17.945	38897	1.33	7606	1.81	5.11 1-Amino-7-guanidino-hept-3-yne
24	18.030	17.945	18.045	34131	1.17	7441	1.77	4.59 4-Chlorobutyric acid, pentadecyl ester
25	18.480	18.465	18.550	23914	0.82	7749	1.85	3.09 Butanal, 3-methyl-
26	19.013	18.990	19.160	28957	0.99	5900	1.41	4.91
27	19.487	19.415	19.505	21700	0.74	6467	1.54	3.36 Histidine, 4-nitro-
28	19.650	19.505	19.660	46623	1.60	8986	2.14	5.19
29	19.866	19.660	20.085	1296186	44.39	141993	33.82	9.13 9,12-Octadecadienoic acid, ethyl ester
30	20.600	20.445	20.750	464528	15.91	61875	14.74	7.51 (E)-9-Octadecenoic acid ethyl ester
31	23.042	22.805	23.265	340040	11.65	22366	5.33	15.20 l-(+)-Ascorbic acid 2,6-dihexadecanoate
				2919710	100.00	419879	100.00	

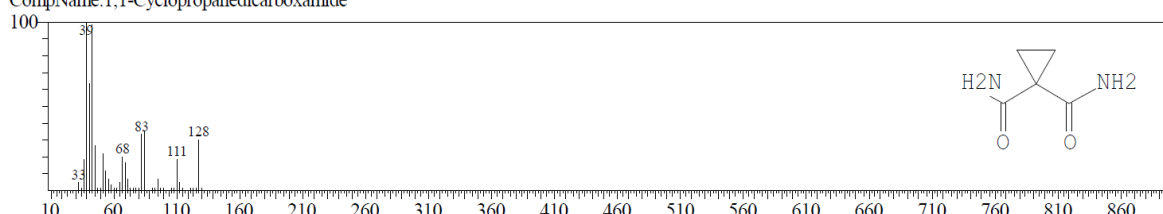
Figure 3: GC-Mass analysis of extract sample



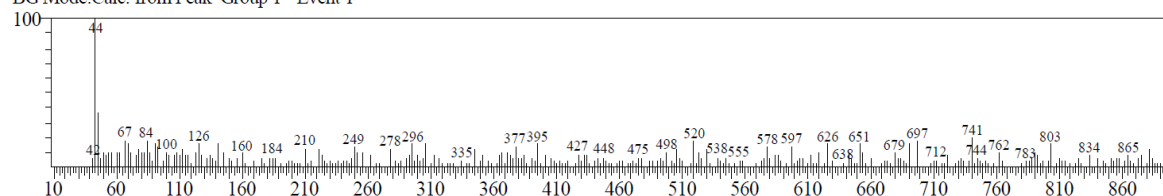
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BG Mode:Calc. from Peak Group 1 - Event 1



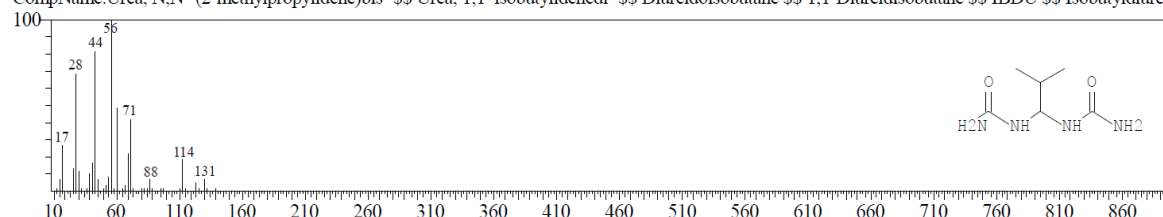
Hit#:1 Entry:6995 Library:NIST11.lib
SI:45 Formula:C5H8N2O2 CAS:5813-85-4 MolWeight:128 RetIndex:1313
CompName:1,1-Cyclopropanedicarboxamide



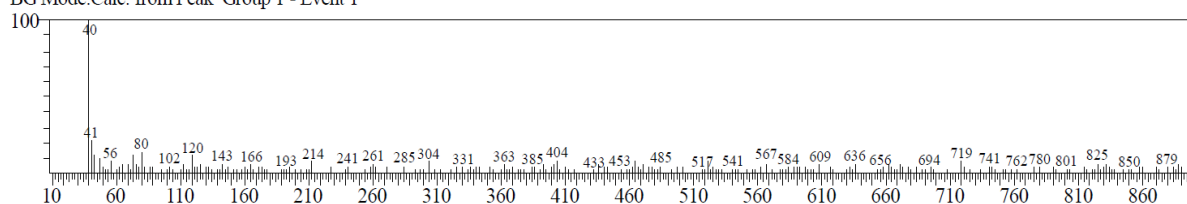
Line#:6 R.Time:5.970(Scan#:595) MassPeaks:486
RawMode:Averaged 5.965-5.975(594-596) BasePeak:44.05(258)
BG Mode:Calc. from Peak Group 1 - Event 1



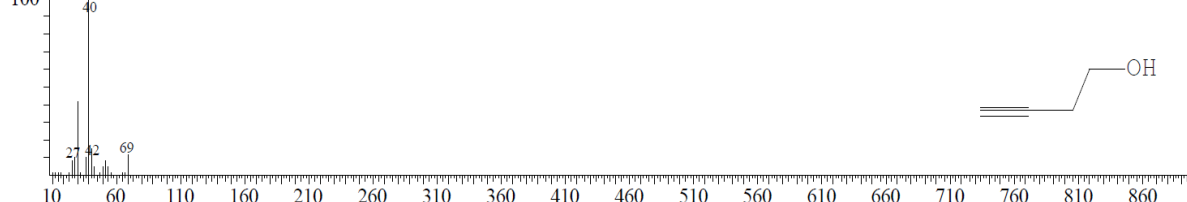
Hit#:1 Entry:27666 Library:NIST11.lib
SI:42 Formula:C6H14N4O2 CAS:6104-30-9 MolWeight:174 RetIndex:1705
CompName:Urea, N,N''-(2-methylpropylidene)bis- \$ Urea, 1,1'-isobutylidenedi- \$ Diureidoisobutane \$ 1,1-Diureidoisobutane \$ IBDU \$ Isobutyldiurea



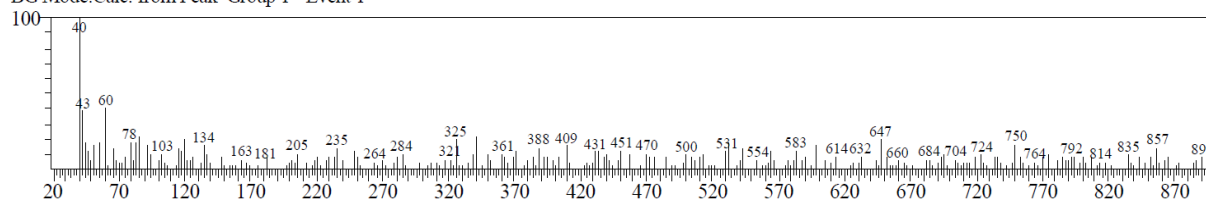
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RawMode:Averaged 6.240-6.250(649-651) BasePeak:40.00(447)
BG Mode:Calc. from Peak Group 1 - Event 1



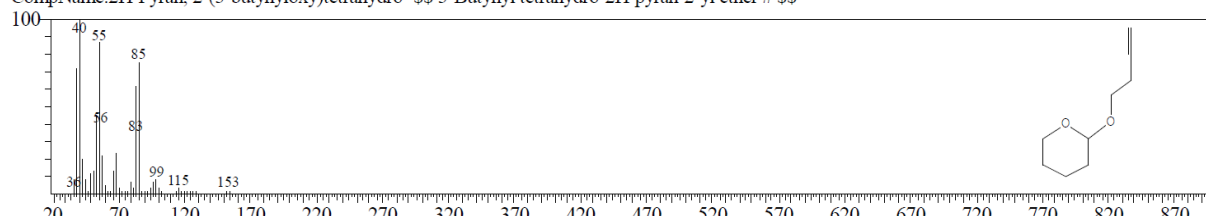
Hit#:1 Entry:249 Library:NIST11.lib
SI:44 Formula:C4H6O CAS:927-74-2 MolWeight:70 RetIndex:659
CompName:3-Butyn-1-ol \$ 1-Butyn-4-ol \$ 2-Hydroxyethylacetylene \$ 3-Butyne-1-ol \$ 3-Butynol \$ 3-Butynyl alcohol \$ 4-Hydroxy-1-butyne \$ HC



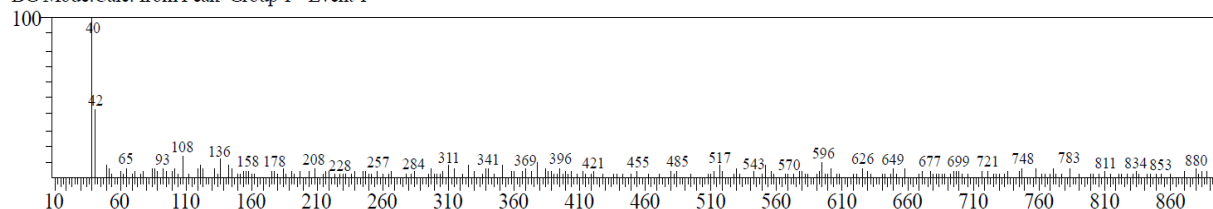
Line#:8 R.Time:8.835(Scan#:1168) MassPeaks:390
RawMode:Averaged 8.830-8.840(1167-1169) BasePeak:40.00(196)
BG Mode:Calc. from Peak Group 1 - Event 1



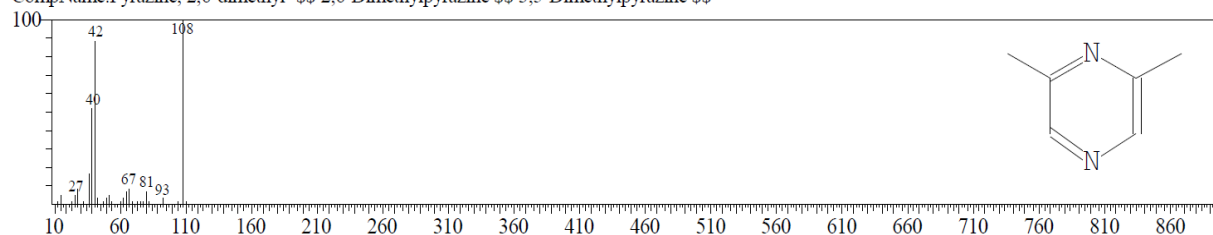
Hit#:1 Entry:17233 Library:NIST11.lib
SI:37 Formula:C9H14O2 CAS:40365-61-5 MolWeight:154 RefIndex:1141
CompName:2H-Pyran, 2-(3-butyloxy)tetrahydro- \$\$ 3-Butynyl tetrahydro-2H-pyran-2-yl ether # \$\$



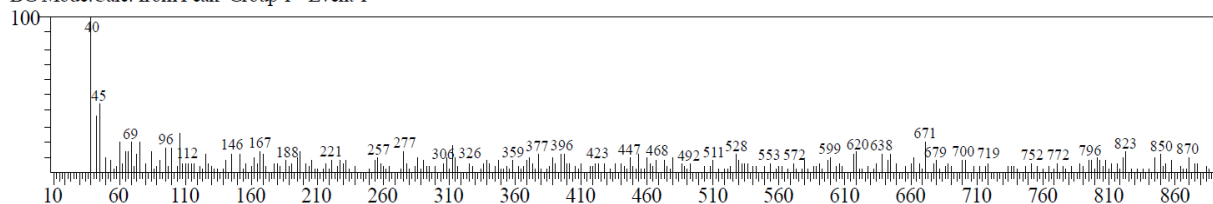
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BG Mode:Calc. from Peak Group 1 - Event 1



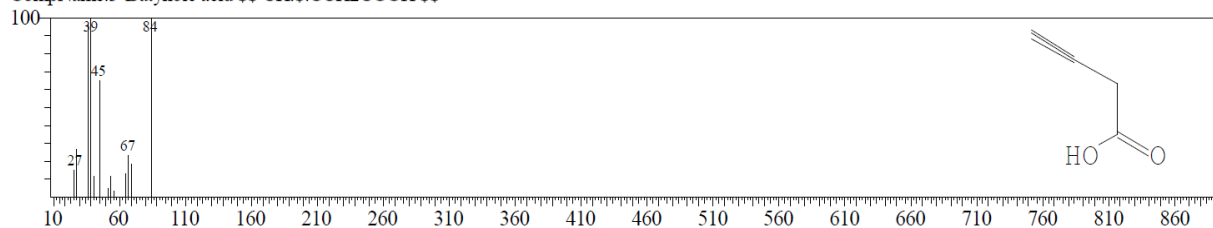
Hit#:1 Entry:2799 Library:NIST11.lib
SI:45 Formula:C6H8N2 CAS:108-50-9 MolWeight:108 RefIndex:894
CompName:Pyrazine, 2,6-dimethyl- \$\$ 2,6-Dimethylpyrazine \$\$ 3,5-Dimethylpyrazine \$\$



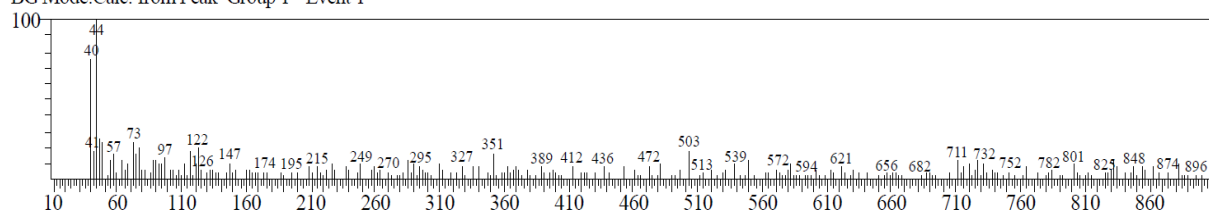
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BG Mode:Calc. from Peak Group 1 - Event 1



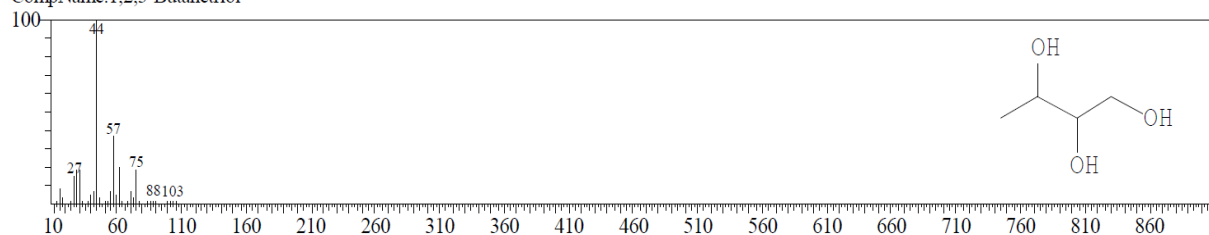
Hit#:1 Entry:652 Library:NIST11.lib
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CompName:3-Butynoic acid \$\$ CH3.CCH2COOH \$\$



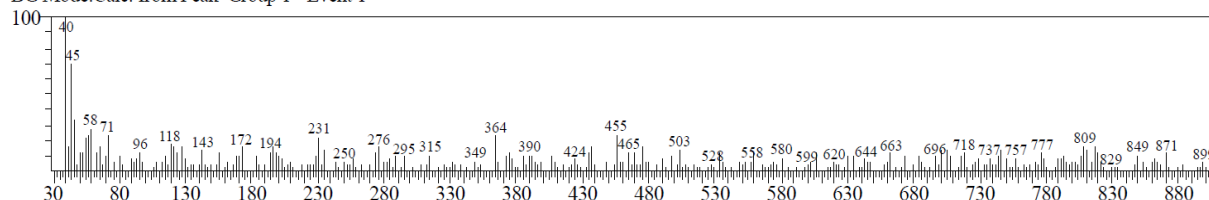
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BG Mode:Calc. from Peak Group 1 - Event 1



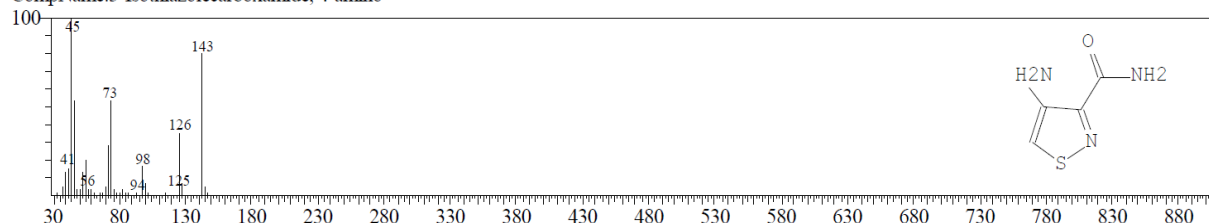
Hit#:1 Entry:2626 Library:NIST11.lib
SI:53 Formula:C4H10O3 CAS:4435-50-1 MolWeight:106 RetIndex:986
CompName:1,2,3-Butanetriol



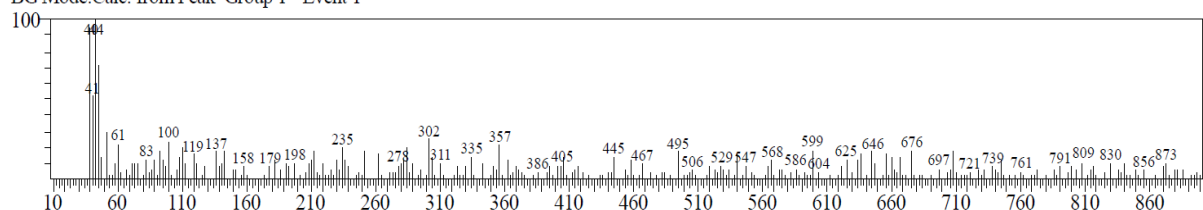
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RawMode:Averaged 10.535-10.545(1508-1510) BasePeak:40.00(197)
BG Mode:Calc. from Peak Group 1 - Event 1



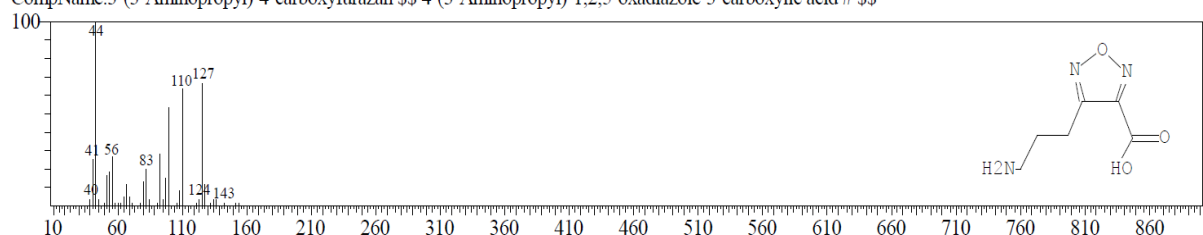
Hit#:1 Entry:12367 Library:NIST11.lib
SI:43 Formula:C4H5N3OS CAS:0-00-0 MolWeight:143 RetIndex:1475
CompName:3-Isothiazolocarboxamide, 4-amino-



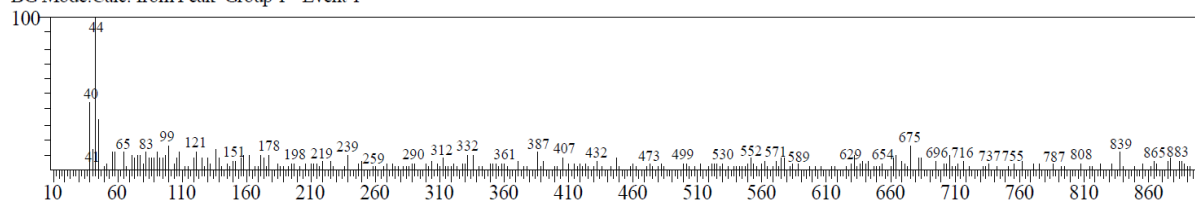
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RawMode:Averaged 11.665-11.675(1734-1736) BasePeak:44.05(147)
BG Mode:Calc. from Peak Group 1 - Event 1



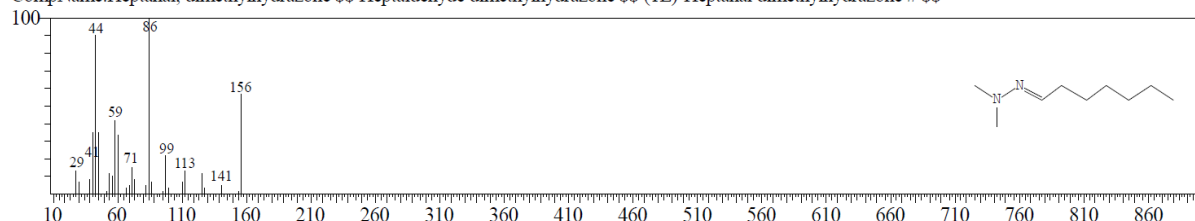
Hit#:1 Entry:26199 Library:NIST11.lib
SI:38 Formula:C6H9N3O3 CAS:0-00-0 MolWeight:171 RetIndex:1606
CompName:3-(3-Aminopropyl)-4-carboxyfurazan 3-(3-Aminopropyl)-1,2,5-oxadiazole-3-carboxylic acid # \$



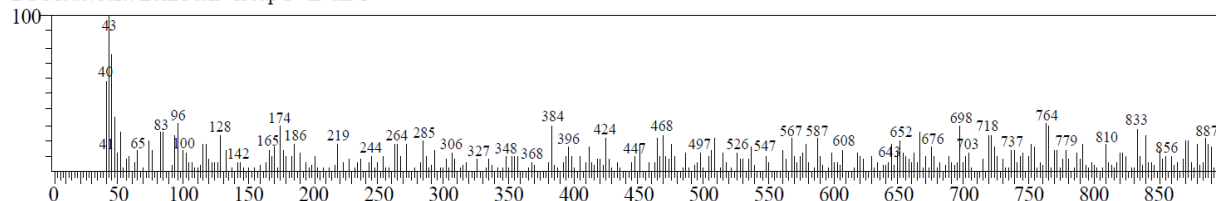
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BG Mode:Calc. from Peak Group 1 - Event 1



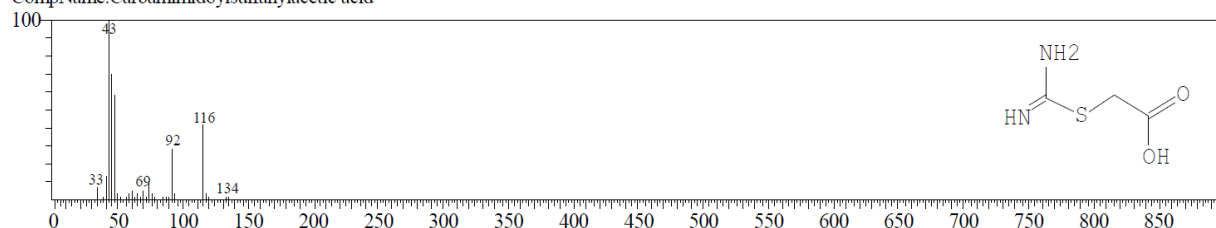
Hit#:1 Entry:18544 Library:NIST11.lib
SI:48 Formula:C9H20N2 CAS:67660-53-1 MolWeight:156 RefIndex:1181
CompName:Heptanal, dimethylhydrazone \$\$ Heptaldehyde dimethylhydrazone \$\$ (1E)-Heptanal dimethylhydrazone # \$\$



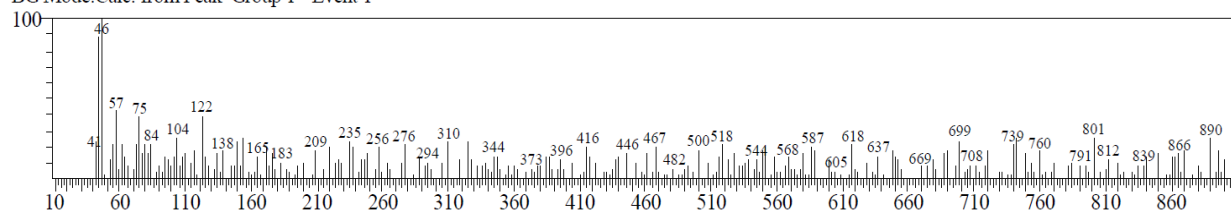
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RawMode:Averaged 13.560-13.570(2113-2115) BasePeak:43.05(130)
BG Mode:Calc. from Peak Group 1 - Event 1



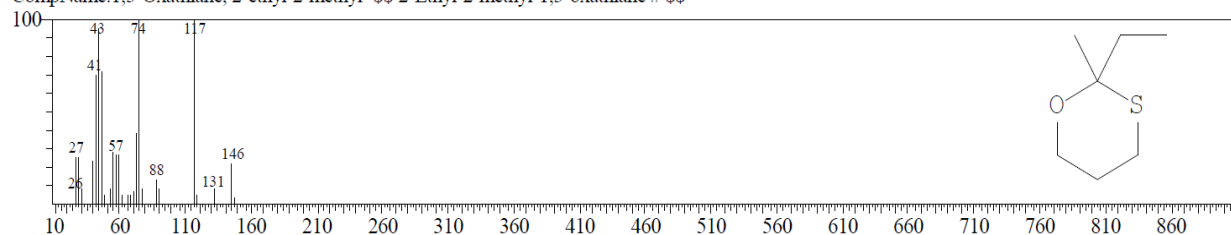
Hit#:1 Entry:8781 Library:NIST11.lib
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CompName:Carbamimidoylsulfanylacetic acid



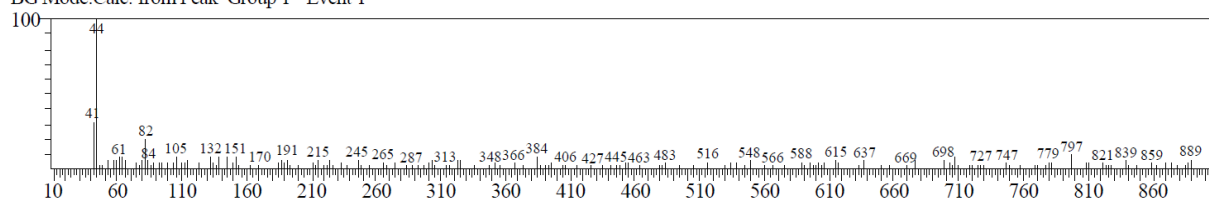
Line#:17 R.Time:14.825(Scan#:2366) MassPeaks:403
RawMode:Averaged 14.820-14.830(2365-2367) BasePeak:46.10(124)
BG Mode:Calc. from Peak Group 1 - Event 1



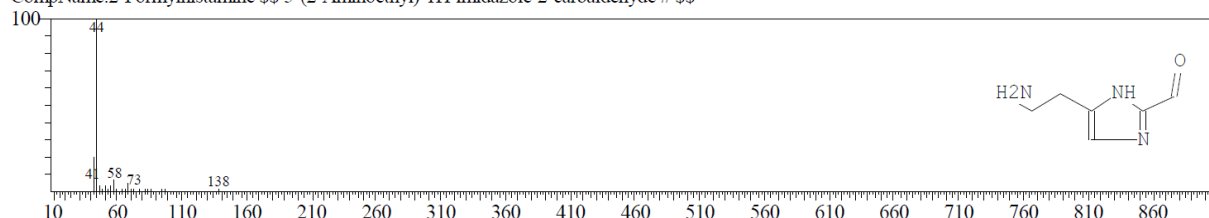
Hit#:1 Entry:13635 Library:NIST11.lib
SI:41 Formula:C7H14OS CAS:30098-80-7 MolWeight:146 RefIndex:1084
CompName:1,3-Oxathiane, 2-ethyl-2-methyl- \$\$ 2-Ethyl-2-methyl-1,3-oxathiane # \$\$



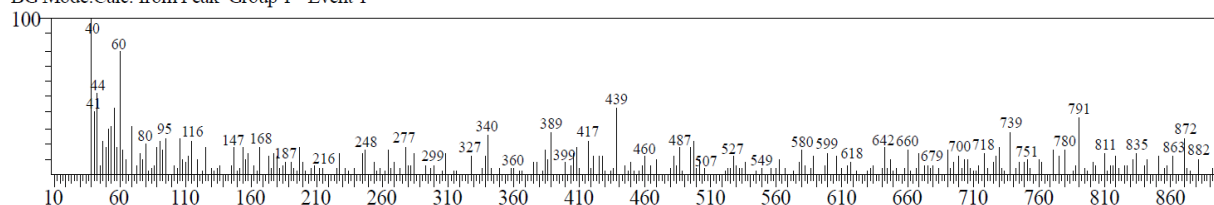
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BG Mode:Calc. from Peak Group 1 - Event 1



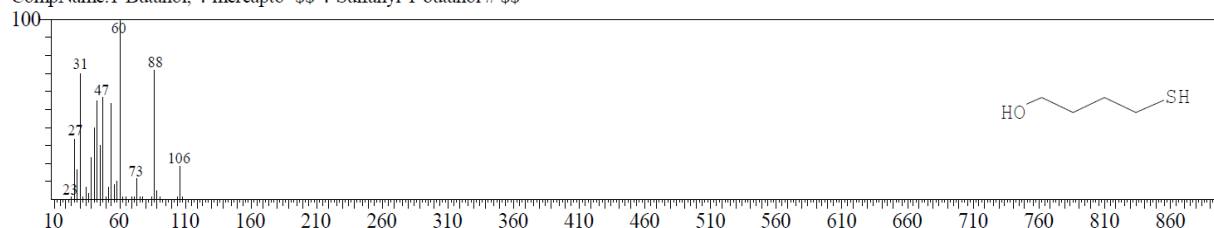
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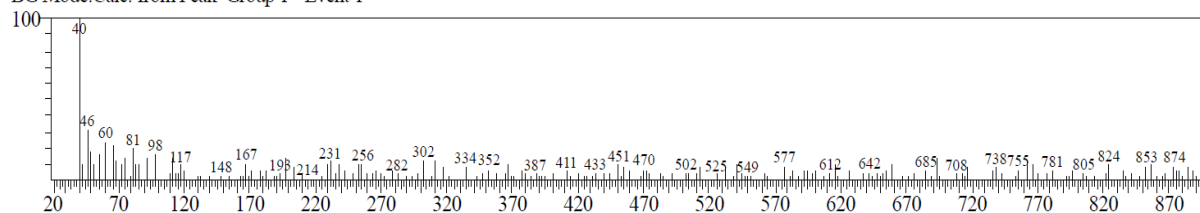
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BG Mode:Calc. from Peak Group 1 - Event 1



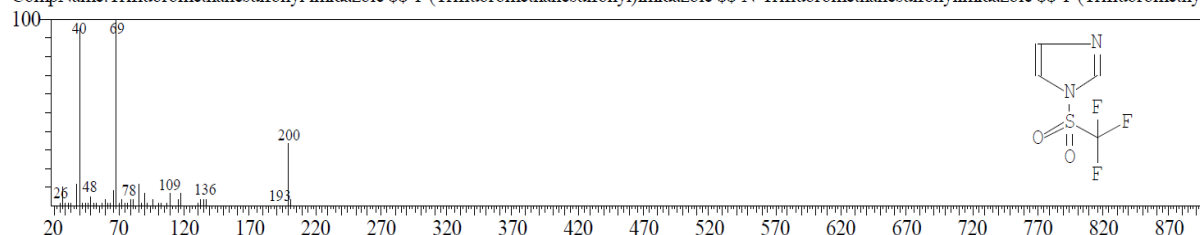
Hit#:1 Entry:2622 Library:NIST11.lib
SI:38 Formula:C₄H₁₀OS CAS:14970-83-3 MolWeight:106 RetIndex:965
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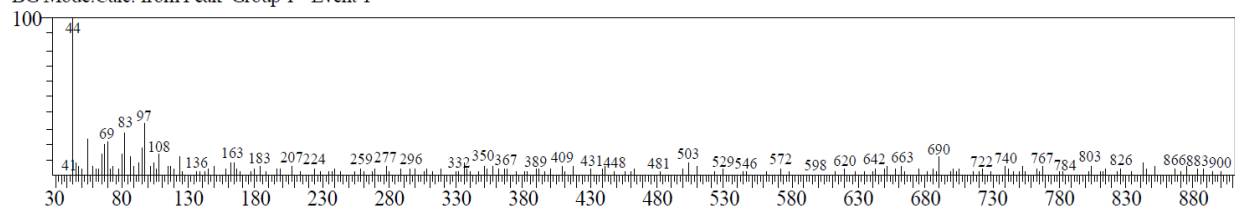
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RawMode:Averaged 16.440-16.450(2689-2691) BasePeak:40.00(244)
BG Mode:Calc. from Peak Group 1 - Event 1



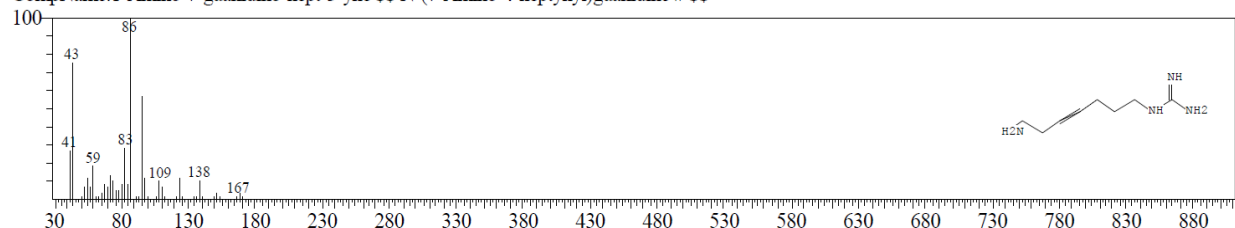
Hit#:1 Entry:43384 Library:NIST11.lib
SI:40 Formula:C₄H₃F₃N₂O₂S CAS:29540-81-6 MolWeight:200 RetIndex:1056
CompName:Trifluoromethanesulfonyl imidazole \$\$ 1-(Trifluoromethanesulfonyl)imidazole \$\$ N-Trifluoromethanesulfonylimidazole \$\$ 1-(Trifluoromethyl



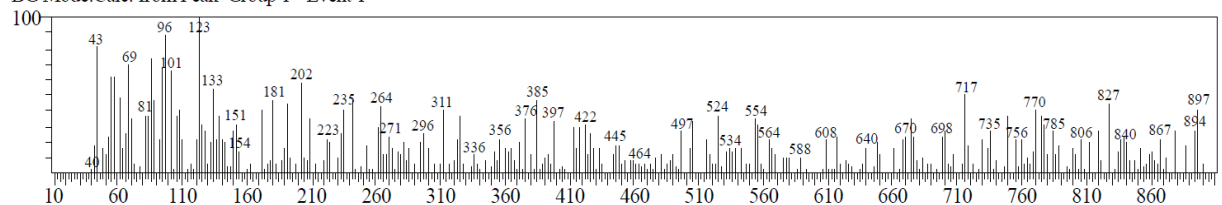
Line#:23 R.Time:17.935(Scan#:2988) MassPeaks:323
RawMode:Averaged 17.930-17.940(2987-2989) BasePeak:44.05(318)
BG Mode:Calc. from Peak Group 1 - Event 1



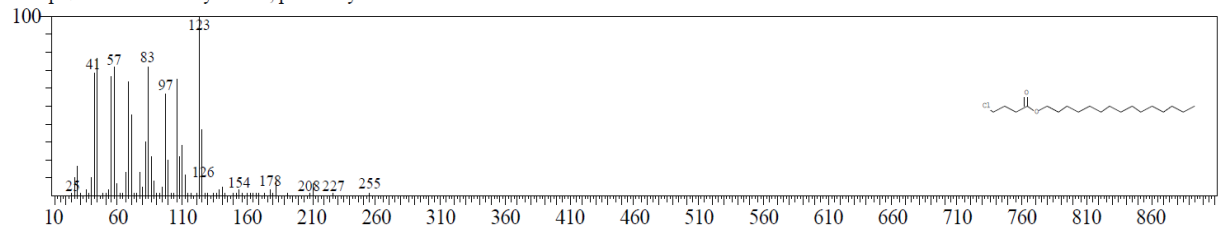
Hit#:1 Entry:24297 Library:NIST11.lib
SI:61 Formula:C8H16N4 CAS:0-00-0 MolWeight:168 RetIndex:1477
CompName:1-Amino-7-guanidino-hept-3-yne \$\$ N-(7-Amino-4-heptynyl)guanidine # \$\$



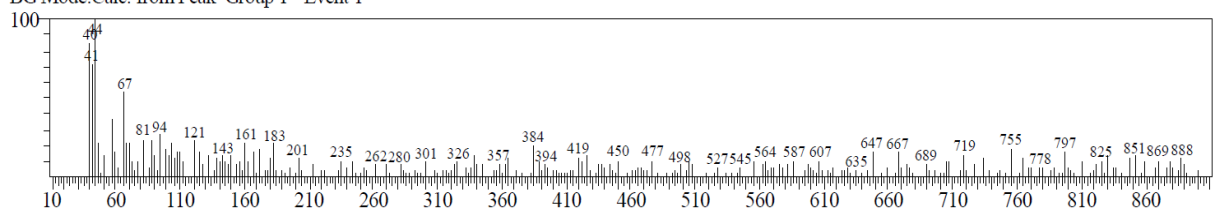
Line#:24 R.Time:18.030(Scan#:3007) MassPeaks:389
RawMode:Averaged 18.025-18.035(3006-3008) BasePeak:123.10(72)
BG Mode:Calc. from Peak Group 1 - Event 1



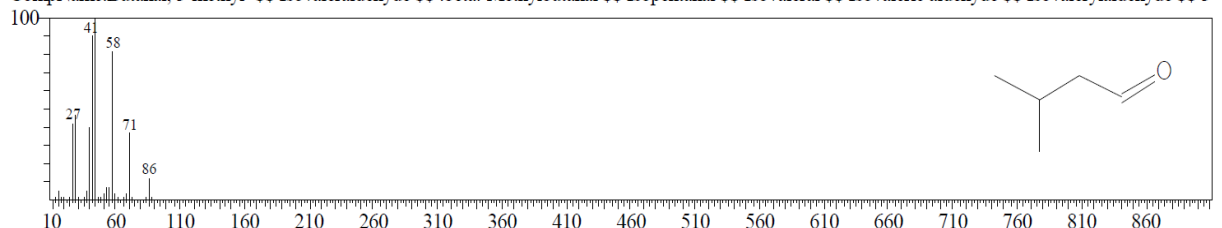
Hit#:1 Entry:143083 Library:NIST11.lib
SI:41 Formula:C19H37ClO2 CAS:0-00-0 MolWeight:332 RetIndex:2302
CompName:4-Chlorobutyric acid, pentadecyl ester



Line#:25 R.Time:18.480(Scan#:3097) MassPeaks:392
RawMode:Averaged 18.475-18.485(3096-3098) BasePeak:44.05(193)
BG Mode:Calc. from Peak Group 1 - Event 1



Hit#:1 Entry:854 Library:NIST11.lib
SI:50 Formula:C5H10O CAS:590-86-3 MolWeight:86 RetIndex:643
CompName:Butanal, 3-methyl- \$\$ Isovaleraldehyde \$\$.beta.-Methylbutanal \$\$ Isopentanal \$\$ Isovaleral \$\$ Isovaleric aldehyde \$\$ Isovalerylaldehyde \$\$ 3



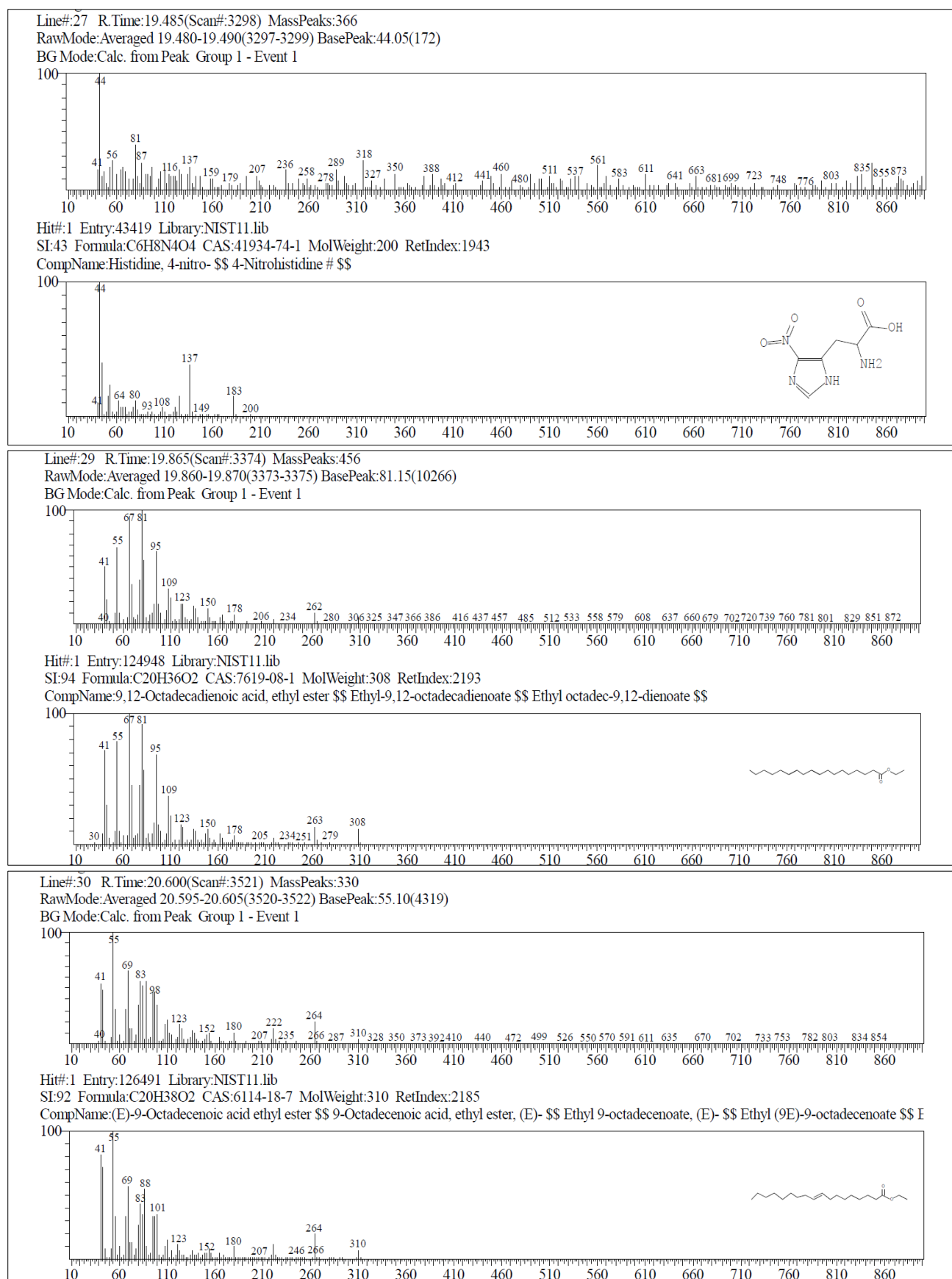


Figure 4: peaks and chemical formula for each compound detected by GC-Mass analysis

The figures detailed above are the 24 compounds peaks resulted from GC-Mass analysis. The results of FTIR and GC-MS analyses illustrated in the figures listed above exhibited a variety of compounds that are produced as a secondary metabolite SM in *A. maurorum* leaves that are grown in the field under different environmental conditions, (24) compounds detected in GC-Mass analysis in different peaks, these metabolites involved in a different physiological and metabolic response in plant body to cope biotic and climatic stresses and maintain survival. The SM protect against the ROS reactive oxygen species that are produced in plants subjected to stressors that cause cellular damage and degradation of proteins and enzymes affect genes that are essential for metabolic pathways (Choudhury *et al.*, 2013). 1,1-Cyclopropanedicarboxamide, 3-Butyn-1-ol, 1,2,3-Butanetriol, stabilizing cellular compartments osmoprotection under salinity and water deficiency 4-Chlorobutyric Acid, Pentadecyl Ester, 9,12-Octadecadienoic Acid, Ethyl Ester, (E)-9-Octadecenoic Acid Ethyl Ester, 1-Butanol, 4-mercapto Lipid related compounds stabilizing membranes, enhancing fluidity, maintain membrane integrity and signals pathways under stresses (Rawat *et al.*, 2021). 1,3,4-Oxadiazole derivatives exhibited promising antimicrobial properties that eliminate biotic threats such as sects and pathogenic bacteria that could harm the plant (Tiwarei *et al.*, 2022; Zhong *et al.*, 2023). 2H-Pyran, 2-(3-butynyloxy) has been reported to exhibit antimicrobial activity against the bacteria *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* (Bennett *et al.*, 2024). 2-Formylhistamine, Butanal, 3-methyl-, 3-(3-Aminopropyl)-4-carboxyfurazan, 1-Amino-7-guanidino-hept-3-yne, ascorbic acid involved in regulating photosynthesis pathway, hormone biosynthesis, and other antioxidants regeneration. Ascorbic acid involved in cell division and growth and signal transduction and other compounds support signaling, detoxification and stabilizing cellular structures under stresses (Gallie, 2013).

Due to the differences in climate conditions among regions, the results obtained in this study were differ significantly from other rare previous studies that focus on the metabolic role of reduced Alhagi leaves in terms of the compounds produced in the leaves and this resorted to the difference climate conditions surrounding the plant species. Climate fluctuations, absence of neighbour plants, genetic variations and plant variety are factors decisive to the SM produced (AE, 2015; Al Allan, 2024; Mohammed & Abd-alkadhemand, 2022; Montesinos-Navarro *et al.*, 2024; Mostafa & Essawy, 2019; Muhammad *et al.*, 2015; Wagay & Rothe, 2016).

CONCLUSION

The study aimed to evaluate the metabolites of *A. maurorum* camel thorn that was grown naturally under fluctuated environmental conditions compared to other studies. The metabolites were strongly affected by the environmental conditions compared to the rare literature that was focused on roots and other parts that neglect the climatic factors surrounding the plants and neighbouring plants. The number and type of compounds detected showed that the reduced leaves exhibit morphological adaptations to cope environment rather than metabolic adaptations compared to other plant parts and compared to the previous studies.

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