CHARACTERIZATION OF PHYTOCHEMICAL CONSTITUENTS IN HEXANE EXTRACTS OF STEVIA REBAUDIANA (LEAVES) BY GC-MS

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Abstract
Studies revealed that stevia represent a new opportunity for researchers and farmers both. It is a natural alternative to artificial sweetener belongs to asteracece family. Hexane extract prepared from stevia leaves and characterized by Gas Chromatography-Mass Spectroscopy in which 29 compounds were identified which showed the presence of acid, ester and hydrocarbon. The presence of different bioactive compounds in the Stevia rebaudiana (leaves) showed the use of plant for various industries and pharmaceuticals.
Key words: Stevia rebaudiana (leaves), chemical compounds, gas chromatography mass spectrometry.

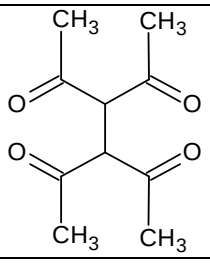
I. INTRODUCTION

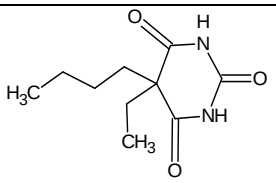
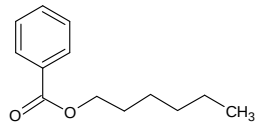
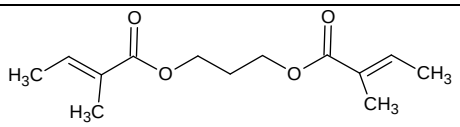
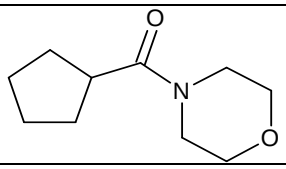
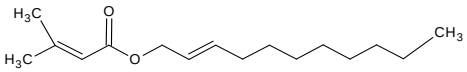
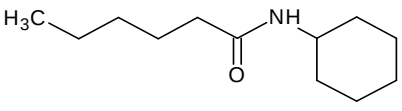
The worldwide demand for high potency sweeteners is increasing, especially with the new practice of blending different sugars. Honey and fruits used as sweeteners in ancient time Cane sugar with beet sugar are the main source of sugar with sweetening qualities, but it also contributes calories that lead to obesity, diabetes mellitus, hypertension, cardiovascular diseases etc [5].

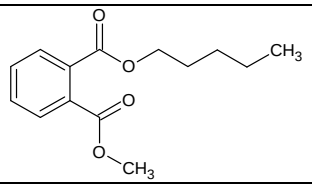
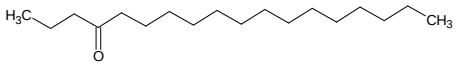
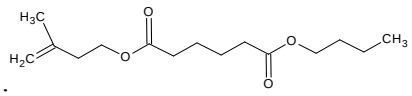
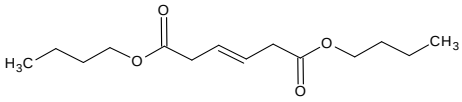
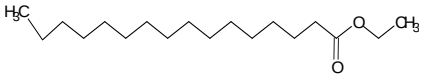
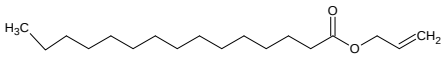
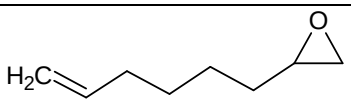
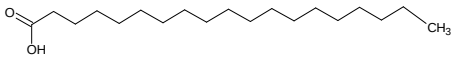
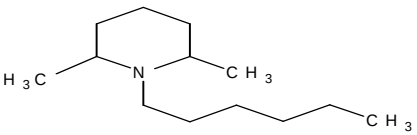
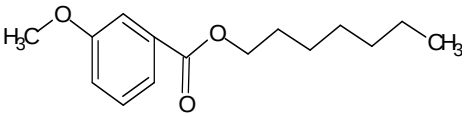
Stevia species are a rich source of biological active molecules including sesquiterpenes, lactones, glycosides, triterpenes and flavonoids [1] [2] with antimicrobial, antifungal, antioxidant, anti-inflammatory and anticarcinogenic activities [3] [4]. Different species of Stevia contain several potential sweetening compounds, with Stevia rebaudiana being the sweetest of all.

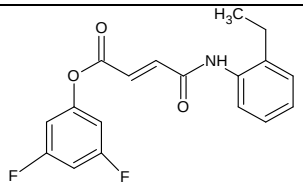
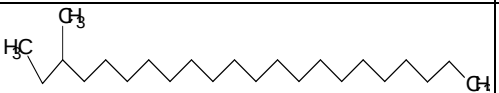
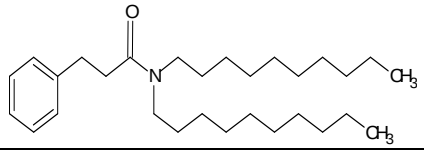
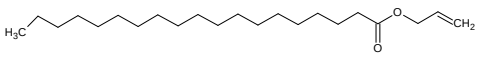
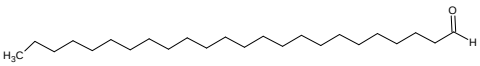
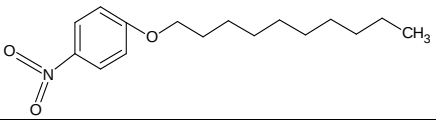
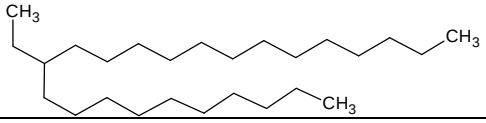
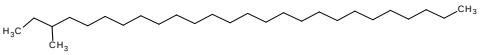
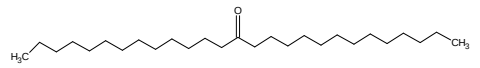
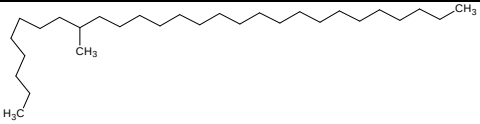
In the present paper, we report the phytochemicals isolated from hexane extract by gas chromatography mass spectrometry (GC-MS).

Table Phytochemicals identified in hexane extracts of Stevia Rebaudiana leaves

Sl. No.	R. Time	Structure of compound	Name of the compound, Molecular formula, Molecular Mass	MS fragment ions	Uses
01.	8.635		1,1,2,2-Tetracetylethane $C_{10}H_{14}O_4$,198	154, 141, 127, 113, 99, 85, 71,57,55	Solid detergent

02.	9.820		Butethal $C_{10}H_{16}N_2O_3$, 212	155,141, 126, 113, 99, 85,71,,57,55	Sedative and a hypnotic drug
03.	10.035		Benzoic acid, hexyl ester $C_{13}H_{18}O_2$, 206	191,175,163, 147, 133,115, 105, 91, 74, 57, 55	Preservatives in pharmaceuticals antimicrobial properties
04.	11.100		Hexadecane $C_{16}H_{34}$, 226	141, 127, 113, 99, 85,71, 57, 55	Anti-inflammatory, beta-oxidant and thermogenic functions
05.	12.465		Propane-1, 3-diyl bis (-2-methylbut-2-enoate) $C_{13}H_{20}O_4$,240	155, 141, 127, 113, 99, 85,71,57,55	Pharmaceuticals
06.	12.540		Cyclopentanecarboxylic acid, morpholide $C_{10}H_{17}NO_2$,183	155, 141, 127, 113, 99, 85, 71, 57, 55	Flavouring agent
07.	13.655		3-Methyl-2-butenoic acid, undec-2-enyl ester $C_{16}H_{28}O_2$,252	224,210, 195,154, 140,125,111,97, 83,57,55	Flavour ingredient
08.	13.735		Octadecane $C_{18}H_{38}$,254	155, 141, 127, 113, 99, 85,71,57,55	Lubricants, transformer oil and anti-corrosion agents
09.	13.855		Hexanamide, N-cyclohexyl $C_{12}H_{23}NO$, 197	183, 178, 155, 141, 127, 113, 99, 85, 71, 57, 55	Unknown

10.	14.315		Methyl pentyl phthalate $C_{14}H_{18}O_4$,250	210, 179, 165, 140, 137, 124, 109, 85, 71, 58, 55	Plasticizers
11.	14.910		Octadecan-4-one $C_{18}H_{36}O$, 268	197, 169, 155, 141, 127, 113, 99, 85, 71, 57, 55	Ingredient in lubricants, resins, perfumes
12.	15.225		Adipic acid, butyl 3-methylbut-3-enyl ester $C_{15}H_{26}O_4$,270	239, 227, 213, 199, 185, 171, 157, 143, 129, 125, 101, 87, 74, 55	Pharmaceuticals
13.	15.650		Dibutyl 2-butenedioate $C_{14}H_{24}O_4$, 256	227, 213, 199, 185, 171, 157, 143, 129, 115, 98, 85, 73, 60, 55	Pharmaceuticals
14.	16.045		Hexadecanoic acid, ethyl ester $C_{18}H_{36}O_2$,284	255, 241, 227, 213, 199, 185, 171,157, 143, 125, 101, 88, 83,55	Pharmaceuticals
15.	16.115		Allyl pentadecanoate $C_{18}H_{34}O_2$,282	211, 197, 183, 169, 155, 141, 127, 113, 99, 85, 71, 57, 55	Pharmaceuticals
16.	17.340		Oxirane, 5-hexenyl- $C_8H_{14}O$, 296	226, 211, 169, 155, 141, 127, 113, 99, 85, 71, 57, 55	Unknown
17.	17.680		Nonadecanoic acid $C_{19}H_{38}O_2$,298	267, 255, 241, 213, 199, 185, 157, 143, 129, 101, 87 ,74 , 55	Laboratory uses
18.	18.410		Piperidine, 1-hexyl-2,6-dimethyl $C_{13}H_{27}N$, 327	312, 281, 269, 225, 183, 154, 140, 126, 125, 111, 97, 83, 55, 53	Unknown
19.	18.460		Benzoic acid, 3-methoxy-, heptyl ester $C_{15}H_{22}O_3$, 310	183, 169, 155, 141, 127, 113, 99, 85, 71, 57, 55	Pharmaceuticals

20.	18.705		Fumaric acid, monoamide, N-(2-ethylphenyl)-, 3, 5-difluorophenyl ester $C_{18}H_{15}F_2NO_3$,331	315, 278, 265, 207, 193, 179, 151, 137, 123, 109, 95, 82 , 68, 55, 53	Pharmaceuticals, laboratory uses
21.	19.495		Docosane, 3-methyl $C_{23}H_{48}$,324	183, 169, 155, 141, 127, 113, 99, 85, 71, 57, 55, 54	Lubricants
22.	20.405		Propanamide, N, N-didecyl-3-phenyl- $C_{29}H_{51}NO$,429	336, 327, 316, 281, 249, 223, 194, 181, 153, 126, 125 , 111, 97, 83, 55, 53	Unknown
23.	20.445		Allyl nonadecanoate $C_{22}H_{42}O_2$,338	281, 207, 183, 169, 155, 141, 127, 113, 99, 85, 71, 57, 55	Pharmaceuticals
24.	21.340		Tetracosanal $C_{24}H_{48}O$,352	211, 197, 183, 169, 155, 141, 127, 113, 99, 85, 71, 57, 55, 54	Laboratory uses
25.	21.850		Decyl 4-nitrophenyl ether $C_{16}H_{25}NO_3$,279	167, 149, 132, 113, 104, 84, 71, 57, 55	Unknown
26.	22.175		Hexadecane, 3-decyl $C_{26}H_{54}$,366	197, 183, 169, 155, 141, 127, 113, 99, 85, 71, 57, 55, 54	Lubricants, resins
27.	22.970		Hexacosane, 3-methyl $C_{27}H_{56}$,380.7	211, 197, 183, 169, 155, 141, 127, 113, 99, 85, 71, 57, 55, 54	Soap aromatic-zation
28.	23.725		14-Heptacosanone $C_{27}H_{54}O$,394.7	207, 183, 169, 155, 141, 127, 113, 99, 85, 71, 57, 55, 54	Food and drink
29.	24.460		Octacosane, 9-methyl $C_{29}H_{60}$,408	225, 211, 197, 183, 169, 155, 141, 127, 113, 99, 85, 71, 57, 55, 54	Lubricants, resins

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Sample Information

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Analyzed	: 4/1/2013 3:59:55 PM
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Sample ID	: RS-1
Injection Volume	: 1.00
Data File	: E:\GC-MS Data\year 2013\APRIL13\010413\RS-1.qgd
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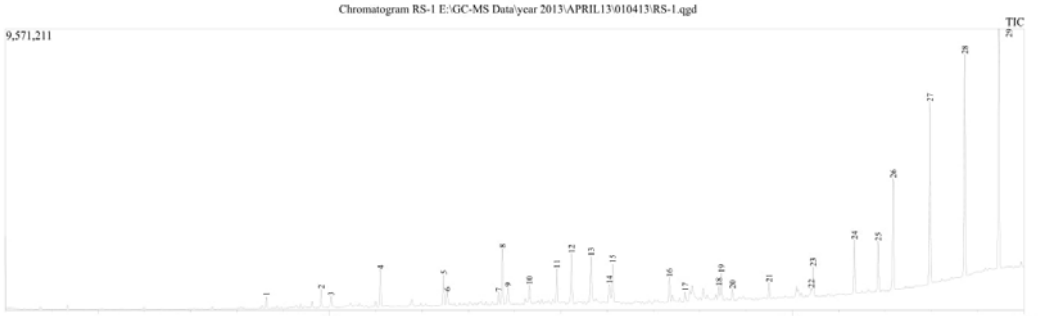
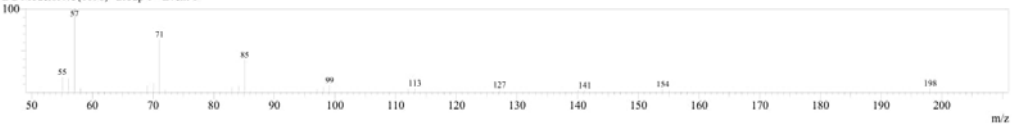
Chromatogram RS-1 E:\GC-MS Data\year 2013\APRIL13\010413\RS-1.qgd

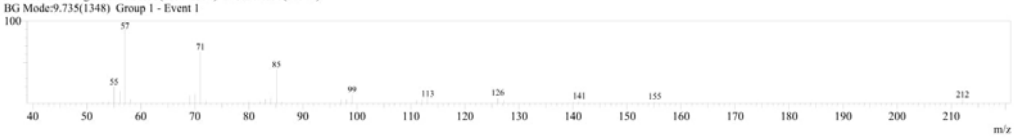


Spectrum

Line#:1 R.Time:8.635(Scan#:1128)
MassPeaks:23
RawMode:Averaged 8.605-8.665(1122-1134) BasePeak:57(46411)
BG Mode:8.475(1096) Group 1 - Event 1



Line#:2 R.Time:9.820(Scan#:1365)
MassPeaks:28
RawMode:Averaged 9.780-9.855(1357-1372) BasePeak:57(57344)
BG Mode:9.735(1348) Group 1 - Event 1



Line#:3 R.Time:10.035(Scan#:1408)
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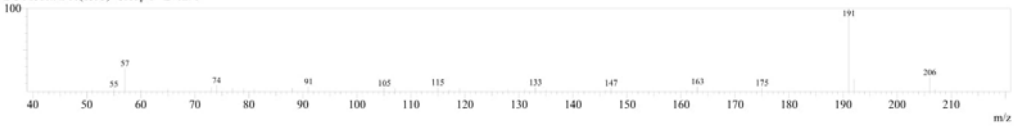
BG Mode:9.960(1393) Group 1 - Event 1



Line#:4 R.Time:11.100(Scan#:1621)
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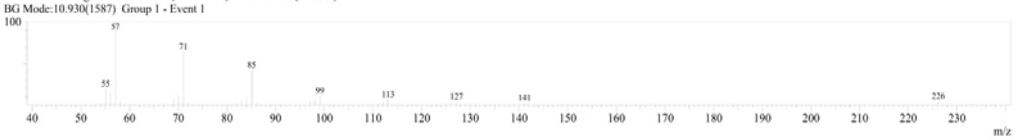
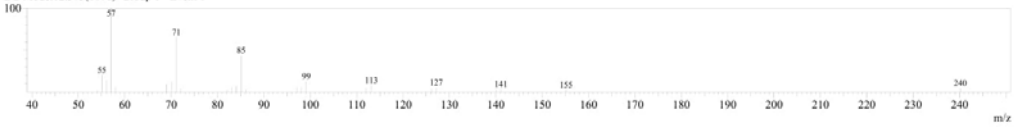


Line#:5 R.Time:12.465(Scan#:1894)
MassPeaks:25
RawMode:Averaged 12.420-12.505(1885-1902) BasePeak:57(96980)
BG Mode:12.375(1876) Group 1 - Event 1



Line#:6 R.Time:12.540(Scan#:1909)
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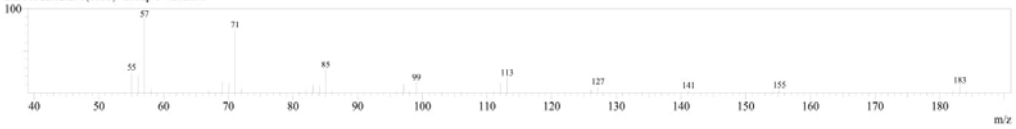
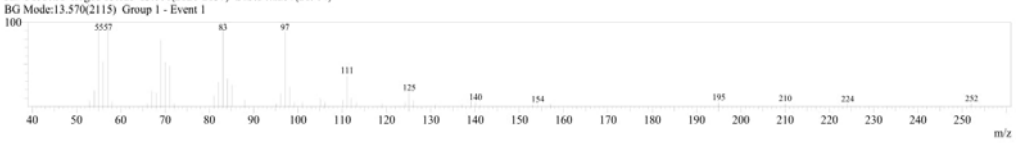
RawMode:Averaged 12.500-12.595(1901-1920) BasePeak:57(43669)
BG Mode:12.270(1855) Group 1 - Event 1



Line#:7 R.Time:13.655(Scan#:2132)
MassPeaks:47
RawMode:Averaged 13.625-13.680(2126-2137) BasePeak:57(21904)
BG Mode:13.570(2115) Group 1 - Event 1



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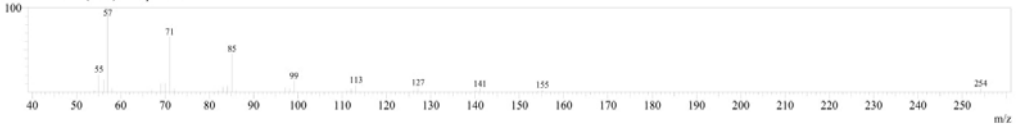

Line#:9 R.Time:13.855(Scan#:2172)
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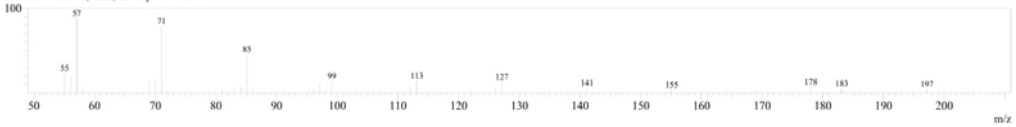

Line#:10 R.Time:14.315(Scan#:2264)
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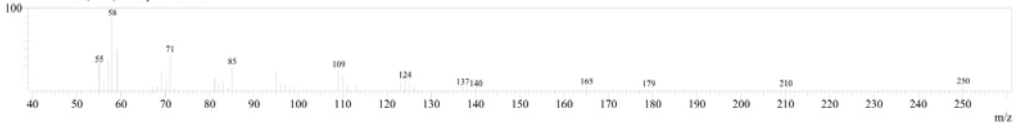
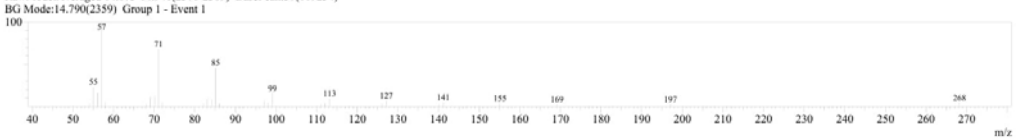
RawMode:Averaged 14.290-14.350(2259-2271) BasePeak:58(40971)
BG Mode:14.275(2256) Group 1 - Event 1

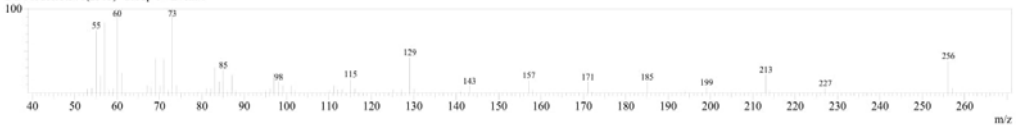


Line#:11 R.Time:14.910(Scan#:2383)
MassPeaks:29
RawMode:Averaged 14.875-14.940(2376-2389) BasePeak:57(119254)
BG Mode:14.790(2359) Group 1 - Event 1



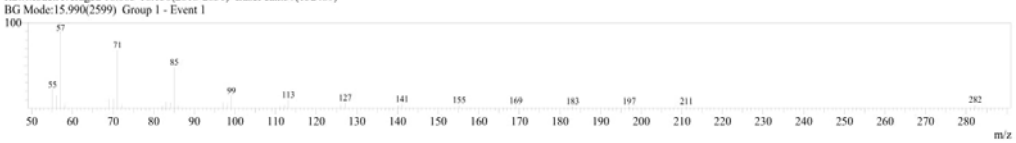
Line#:12 R.Time:15.225(Scan#:2446)
MassPeaks:39
RawMode:Averaged 15.180-15.270(2437-2455) BasePeak:74(126527)
BG Mode:15.105(2422) Group 1 - Event 1

Line#:13 R.Time:15.650(Scan#:2531)
MassPeaks:57
RawMode:Averaged 15.595-15.690(2520-2539) BasePeak:73(56994)
BG Mode:15.570(2515) Group 1 - Event 1

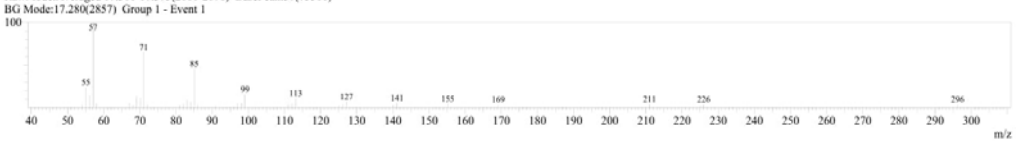


Line#:14 R.Time:16.045(Scan#:2610)
MassPeaks:60
RawMode:Averaged 16.000-16.080(2601-2617) BasePeak:88(27391)
BG Mode:15.970(2595) Group 1 - Event 1

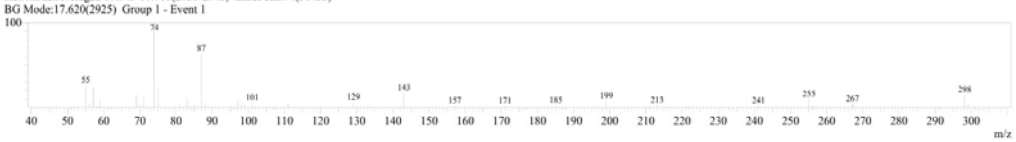
Line#:15 R.Time:16.115(Scan#:2624)
MassPeaks:29
RawMode:Averaged 16.085-16.150(2618-2631) BasePeak:57(152489)
BG Mode:15.990(2599) Group 1 - Event 1



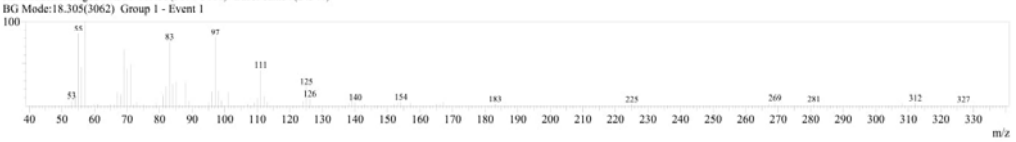
Line#:16 R.Time:17.340(Scan#:2869)
MassPeaks:36
RawMode:Averaged 17.300-17.375(2861-2876) BasePeak:57(75511)
BG Mode:17.280(2857) Group 1 - Event 1



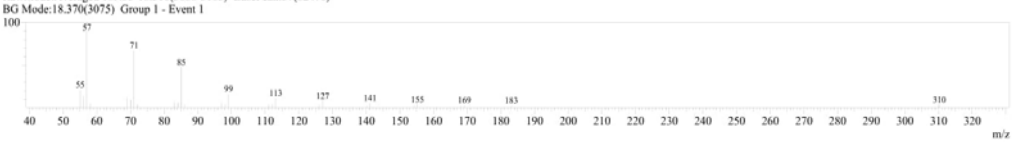
Line#:17 R.Time:17.680(Scan#:2937)
MassPeaks:37
RawMode:Averaged 17.645-17.710(2930-2943) BasePeak:74(36453)
BG Mode:17.620(2925) Group 1 - Event 1



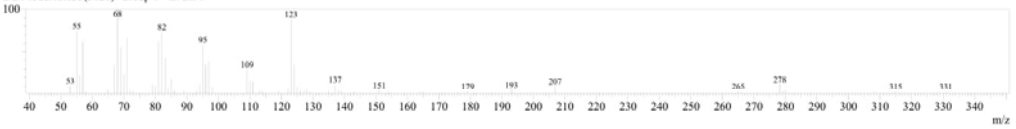
Line#:18 R.Time:18.410(Scan#:3083)
MassPeaks:60
RawMode:Averaged 18.380-18.435(3077-3088) BasePeak:57(24341)
BG Mode:18.305(3062) Group 1 - Event 1



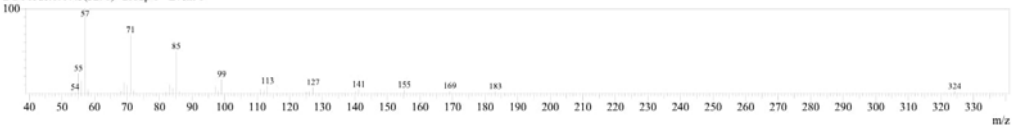
Line#:19 R.Time:18.460(Scan#:3093)
MassPeaks:28
RawMode:Averaged 18.435-18.510(3088-3103) BasePeak:57(82470)
BG Mode:18.370(3075) Group 1 - Event 1



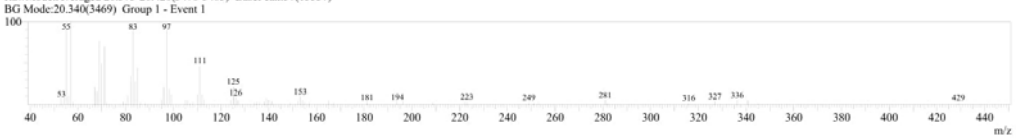
Line#:20 R.Time:18.705(Scan#:3142)
MassPeaks:61
RawMode:Averaged 18.665-18.730(3134-3147) BasePeak:68(13964)
BG Mode:18.635(3128) Group 1 - Event 1



Line#:21 R.Time:19.495(Scan#:3300)
MassPeaks:30
RawMode:Averaged 19.465-19.525(3294-3306) BasePeak:57(58151)
BG Mode:19.445(3290) Group 1 - Event 1



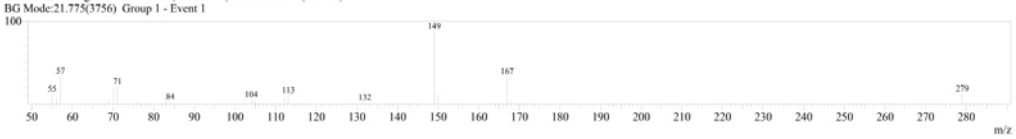
Line#:22 R.Time:20.405(Scan#:3482)
MassPeaks:70
RawMode:Averaged 20.375-20.420(3476-3485) BasePeak:57(15537)
BG Mode:20.340(3469) Group 1 - Event 1



Line#:23 R.Time:20.445(Scan#:3490)
MassPeaks:32
RawMode:Averaged 20.420-20.480(3485-3497) BasePeak:57(102046)
BG Mode:20.285(3458) Group 1 - Event 1

Line#:24 R.Time:21.340(Scan#:3669)
MassPeaks:34
RawMode:Averaged 21.290-21.380(3659-3677) BasePeak:57(127166)
BG Mode:21.235(3648) Group 1 - Event 1

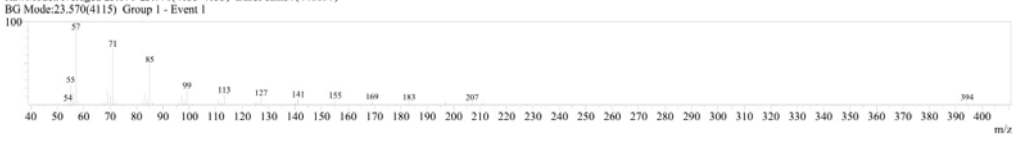
Line#:25 R.Time:21.850(Scan#:3771)
MassPeaks:20
RawMode:Averaged 21.820-21.880(3765-3777) BasePeak:149(227479)
BG Mode:21.775(3756) Group 1 - Event 1



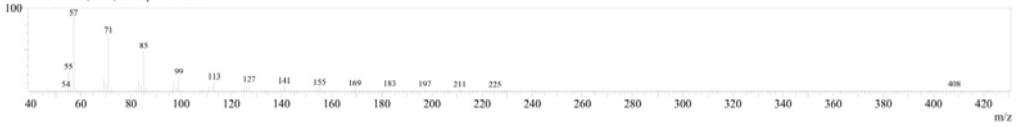
Line#:26 R.Time:22.175(Scan#:3836)
MassPeaks:34
RawMode:Averaged 22.120-22.235(3825-3848) BasePeak:57(203538)
BG Mode:22.065(3814) Group 1 - Event 1

Line#:27 R.Time:22.970(Scan#:3995)
MassPeaks:35
RawMode:Averaged 22.905-23.015(3982-4004) BasePeak:57(327860)
BG Mode:22.830(3967) Group 1 - Event 1

Line#:28 R.Time:23.725(Scan#:4146)
MassPeaks:36
RawMode:Averaged 23.670-23.770(4135-4155) BasePeak:57(448697)
BG Mode:23.570(4115) Group 1 - Event 1



Line#:29 R.Time:24.460(Scan#:4293)
MassPeaks:36
RawMode:Averaged 24.410-24.515(4283-4304) BasePeak:57(478113)
BG Mode:24.325(4266) Group 1 - Event 1



II. MATERIALS AND METHODS

A. Plant material and Extract preparation

The dried leaves of *S. rebaudiana* were purchased from Sun fruit Pvt. Ltd Pune. The leaves of *S. rebaudiana* were shade dried, powdered and extracted in soxhlet extractor serially with n-hexane, dichloro methane, ethyl acetate, acetone, methanol, ethanol and water. Removal of solvent under reduced pressure afforded solid extracts. The removal of solvent under reduced pressure by rotary film evaporator yielded 5gm of hexane extract. As the yield of hexane extract is not good so it is not separated by column chromatography and analysed by GC-MS.

B. GC-MS analysis

Instrument and chromatographic conditions

A Hewlett-Packard 5890 Series II Chromatograph equipped with a flame ionization detector (FID) detector and HP-2 fused silica columns (25 m × 0.32 mm, 0.25 μm film thicknesses) was used. The samples, dissolved in hexane, were injected in the split less mode into helium carrier gas. Injector and detector temperatures were maintained at 250°C. The column temperature was programmed from 60 (after 2 min) to 220°C at

4°C min -1, and the final temperature was held for 20 min. Peak areas and retention times were measured by electronic integration of computer. The relative amounts of individual components are based on the peak areas obtained, without FID response factor correction. GC-MS analyses were carried out on a Hewlett-Packard 5970A mass selective detector (MSD), directly coupled to HP 5790A gas chromatograph. A 26 m × 0.22 mm column, coated with 0.13 μm of CP-Sil 5CB was employed, using helium carrier gas. The oven temperature program was 60°C (3 min), then 5°C min -1 to 250°C (30 min). Electron ionization (EI) mass spectra were acquired over a mass range of 10 to 400 Da at a rate of 2 s-1.

C. Identification of compounds

Compounds were identified by comparing mass spectra data of samples with those of the NIST (National Institute of Standards and Technology, USA) standard reference database. The quantitative estimation of each peak obtained in GC was made by computer, attached with GC-MS instrument. Literature reports already published, also helped in understanding the structure, as well as by comparison of the fragmentation patterns of the compounds present in stevia rebaudiana [6] [7] [8].

III. RESULTS AND DISCUSSION

Different Phytoconstituents have been analysed in stevia species including saturated fatty acid, ester and hydrocarbon, which are used in various industries and ailments such as solid detergent, flavouring agent, lubricants, transformer oils, anti-corrosion agents, plasticizers, ingredient in resins, perfumes. Sedative, hypnoticdrug, preservatives in pharmaceuticals, antimicrobial, anti-inflammatory. However, identification of individual phytochemical constituents and subjecting it to the biological activity will definitely give fruitful results. From the results, it could be concluded that contains various bioactive phytocomponents. Therefore, it can be used as a plant of industrial and pharmaceutical importance.

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