

GC-MS analysis of Iraqi *Silybum marianum* Flowers, Leaves and Seeds Extracts

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Abstract:

Iraqi land is rich with medicinal plants and Iraqi people trust using herbals as treatments since ancient times, one of these important wild medicinal plants is *Silybum marianum*, which is known as Milk thistle. It has a long history in Greek and Arabian medicine as a liver tonic

which has been confirmed by scientific in vivo/ in vitro study and made the plant in the top of medicine that can regenerate liver tissues and cure various liver diseases. The aim of this study is to investigate the active constituents in the seeds, leaves and the flowers of Iraqi *Silybum marianum*, identification have been done by GC-MS instrument after the extraction process by hexane then ethanol as a solvent in the Soxhlet apparatus, An important compounds has been detected such as phytosterol, catechin, terpenes, fatty acids, fatty alcohol, and monosaccharide, in different concentration in the parts of the plants.

Key words: *Silybum marianum*, Milk thistle, GC-MS, phytosterol, catechin, terpene, fatty acids, fatty alcohol, monosaccharide.

أستخدام تقنية كروماتوغرافيا الغاز-مطياف الكتلة لتحليل مستخلصات أوراق و أزهار وبذور

نبات الكلغان العراقي

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الخلاصة:

ان الاراضي العراقية غنية بالنباتات الطبية ومنذ القدم يثق الشعب العراقي بالاستشفاء بالنباتات الطبية. ومن أهم هذه النباتات الطبية النامية بصورة طبيعية في انحاء العراق هو نبات الكلغان او مايعرف ب(شوك الحليب أو السيليمارين)، حيث استخدم كثيرا كمقوي للكبد في الطب العربي والاعريقي القديم والذي اسند لاحقا بالدراسات العلمية والسريرية مما جعل النبات في قمة النباتات المستخدمة في تجديد خلايا الكبد ومعالجته من الامراض. أن الهدف من هذه الدراسة هو تشخيص المركبات الفعالة الموجودة في أوراق و أزهار وبذور النبات، حيث تم أستخلاصها بواسطة جهاز السوكليت بأستخدام الهكسان والايثانول كمذيب ومن ثم تحليل المركبات بواسطة جهاز تحليل كروماتوغرافيا الغاز-مطياف الكتلة حيث تم تشخيص العديد من المركبات الفعالة المهمة كالتربينات، الكحولات الدهنية، الاحماض الدهنية المشبعة وغير المشبعة، السكريات الاحادية، الفايستولول والكاتيكين بنسب مختلفة في أجزاء النبات.

الكلمات المفتاحية: الكلغان، شوك الحليب، كروماتوغرافيا الغاز-مطياف الكتلة، فايستولول، كاتيكين، تربينات، أحماض دهنية، سكريات أحادية

Introduction

History of plant used in the treatment of disease is very ancient that belong to about

sixty thousand years ago in every culture and all countries [1]. From plant species present choosing the right plant, the time of collection of the right part beside the

method of extraction is very important because the active constituents within the same species differ responding to different weather, soil environment, level of growth and even diurnal factors [2]. Nowadays, medicinal plants prescribed in approximately all pharmacopoeias, to use as self-medication or prescribed by a doctor or pharmacist. They are indicated alone or as adjuvant therapy [3]. For adjusting their medicinal medication a lot of studies and trials starting from the quality of the raw constituents passing through formulation and animal studies ending with clinical studies are required [4]. Iraqi land is rich with medicinal plants and Iraqi people trust using herbal as treatments since ancient times as noticed by the Sumerian and Babylonian clay [5]. Geographical and weather diversity in Iraq lead to a variety in plant species and a wide range of local good health concern unit [6]. One of these important wild medicinal plants is *Silybum marianum*, actually it's one of the top selling supplements in the united states that provides income of approximately 3000000 dollars every year. Worldwide philosophers, physicians, scientists, botanists, and pharmacists had written about milk thistle effects on liver, kidney, bile and other diseases [7]. *S. marianum* had been distributed in America, Europe, and other countries from its native Mediterranean region because it can grow in any conditions even in poor nutrient, water and soil need and because of its widely using in diets and medicine as a curing supplement against Amanita mushroom toxins, hepatotoxic elements, viral infections, inflammation and cytotoxic agents [8]. Milk thistle has a long history in Greek and Arabian medicine as a tonic of liver, which has been confirmed by scientific in vivo/ in vitro studies and made the plant in the top of medicine that can regenerate liver tissues and cure various liver diseases [9]. The aim of this study is identification of the active constituents, and its percentage present in

hexane and ethanol extracts for seeds, leaves and flowers of Iraqi *Silybum marianum*.

Experimental

Materials and methods

The solvents and chemicals used for the extraction and derivatization process were received from the commercial suppliers (Iraq, BDH-England, CHEM-LAB Belgium and Merck-Germany).

All the parts of the plant were collected from the college of pharmacy /al-Mustansiriyah University-Qadisiyah/ Baghdad, the plant was authenticated by Dr. Sukaina Abas, Assistant professor in the college of science/ university of Baghdad. They were washed thoroughly by tap water, dried in shade at room temperature from 2 weeks for flowers and seeds up to 1 month for leaves, then grinded to a powder material for further investigation.

Method of extraction

25 grams of shade-dried pulverized flowers were packed in the thimble of Soxhlet apparatus and extracted by (300 mL) of hexane until exhaustion about 24 hours. The extract was filtered then solvent was evaporated by rotary evaporator at 45°C.

The mark was then extracted by (300 mL) of ethanol about 72 hours.

The extract was filtered and evaporated by rotary evaporator at 45°C, the same procedure was repeated to the leaves and the seeds of the plant.

GC-MS analysis of hexane extracts

For active constituents' detection, one microliter of the hexane extract was injected into GC-MS instrument (SHIMADZU LAB) at 250°C. Helium was used as carrier gas and the column temperature was risen up from (80°C to 310°C) at rate time of 10°C/minute [10].

Derivatization and GC-MS analysis of ethanolic extract

Silylation reaction was used to make a derivative by using 1 mL of pyridine to dissolve 10 mg of the dried ethanolic extract, then 0.1 mL of trimethylchlorosilane and 0.2 mL of hexamethyldisilazane was added with stirring, after 5 min the mixture was centrifuged for 5 min at 5000 rpm, one microliter of the supernatant was injected into GC-MS instrument, helium was used as carrier gas and the column temperature was risen up from (80°C to 310°C) at rate time of 10 °C/ minute ^[11].

Results and Discussion

Fatty acids, fatty alcohol and terpene detected by GC-MS in *Silybum marianum* leaves, flowers and seeds

The chromatogram Fig (1,2,3) show important compounds detected in flowers, leaves and seeds hexane extracts respectively compared with database such as terpenes, saturated and unsaturated fatty acids and fatty alcohol; as shown below in table (1) and mass spectrum of each compound shown in Fig (4,5,6,7,8,9,10,11,and

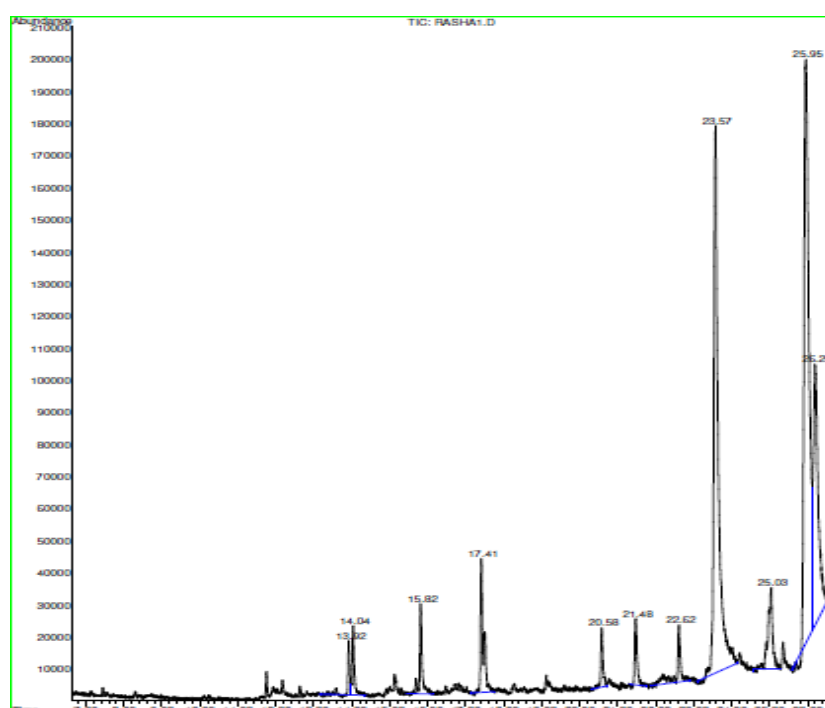


Figure (1) GC-MS chromatogram of hexane extract of *Silybum marianum* flowers

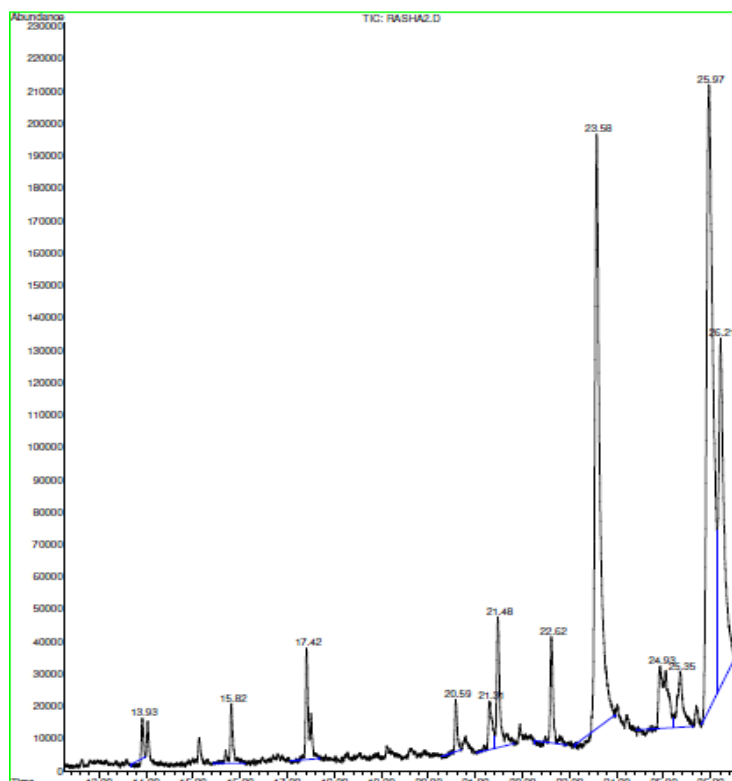


Figure (2): GC-MS chromatogram of hexane extract of *Silybum marianum* leaves

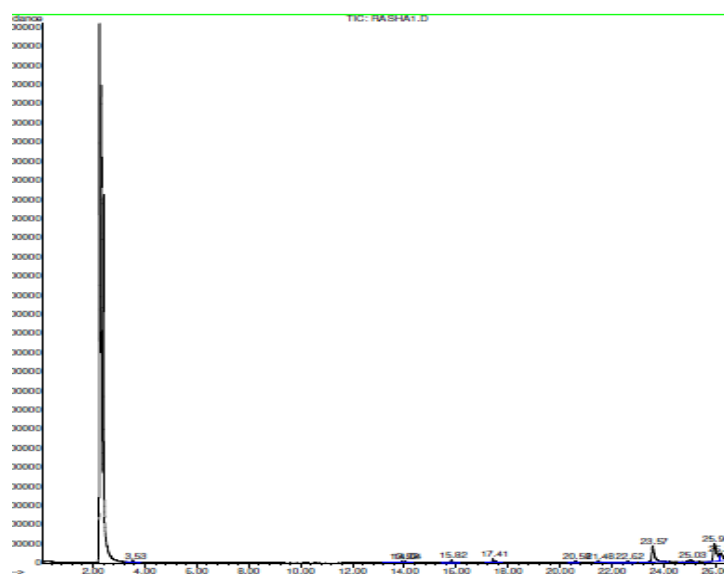
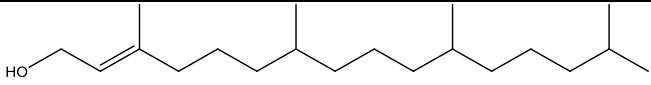
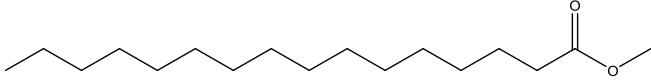
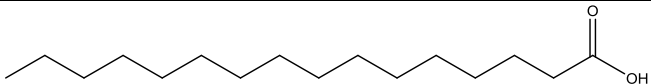

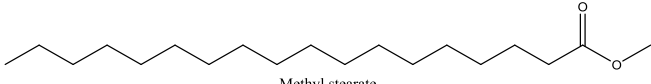
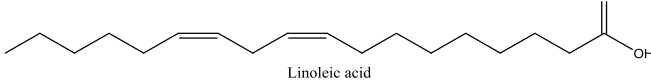
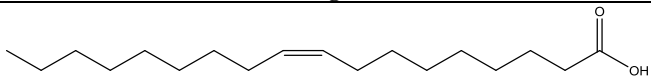
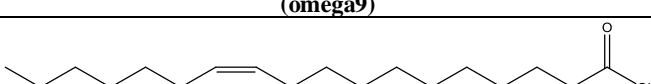
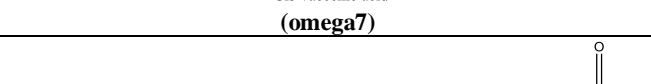


Figure (3): GC-MS chromatogram of hexane extract of *Silybum marianum* seeds

Table (1): Fatty acid, fatty alcohol and diterpene present in *Silybum marianum* leaves, seeds and flowers hexane extract

| Compound | Retention time | Area | | |
|--|----------------|--------|-------|---------|
| | | leaves | seeds | flowers |
|  <p>phytol</p> | 21.31 | 1.73% | - | - |
|  <p>Methyl palmitate (hexadecanoic acid methyl ester)</p> | 22.62 | 2.63% | 0.75% | 2.59% |
|  <p>Palmitic acid</p> | 23.58 | 25.6% | - | 29.26% |
|  <p>Behenyl alcohol (docosanol)</p> | 24.93 | 4.35% | - | - |
|  <p>Methyl stearate</p> | 25.35 | 2.79% | 0.62% | 4.46% |
|  <p>Linoleic acid (omega 6)</p> | 25.95 | 1.99% | 9.88% | 33.3% |
|  <p>Oleic acid (omega9)</p> | 25.97 | 35.15% | 33.7% | 0.87% |
|  <p>Cis-vaccenic acid (omega7)</p> | 26.21% | - | - | 15.5% |
|  <p>Stearic acid</p> | 26.22% | 15.45% | - | - |

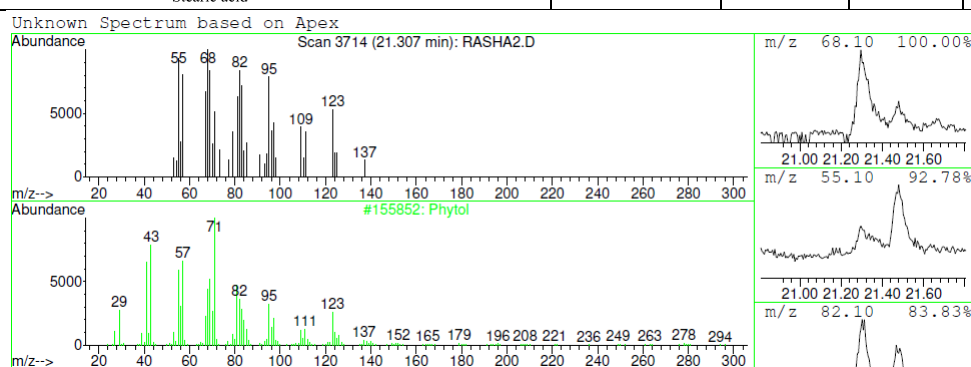


Figure (4): Mass fragment of phytol

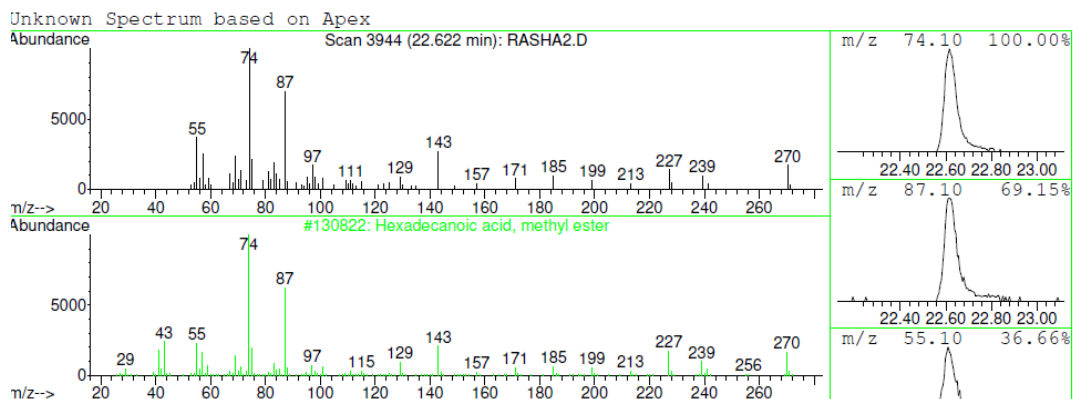


Figure (5): Mass fragment of methyl palmitate (hexadecanoic acid, methyl ester)

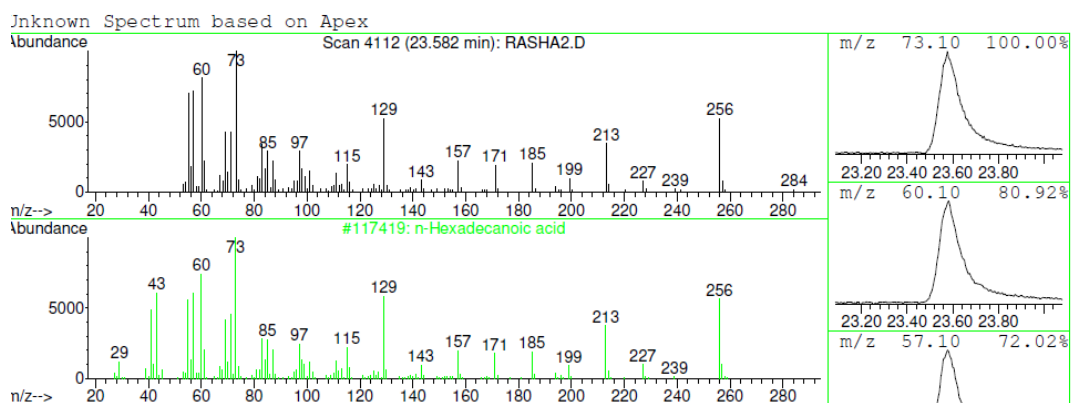


Figure (6): Mass fragment of n-hexadecanoic acid (palmitic acid)

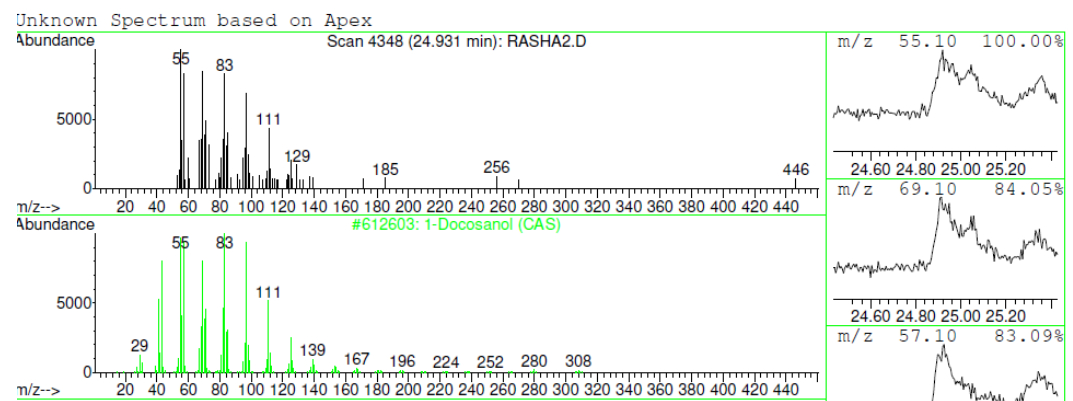


Figure (7): Mass fragment of 1-docosanol (behenyl alcohol)

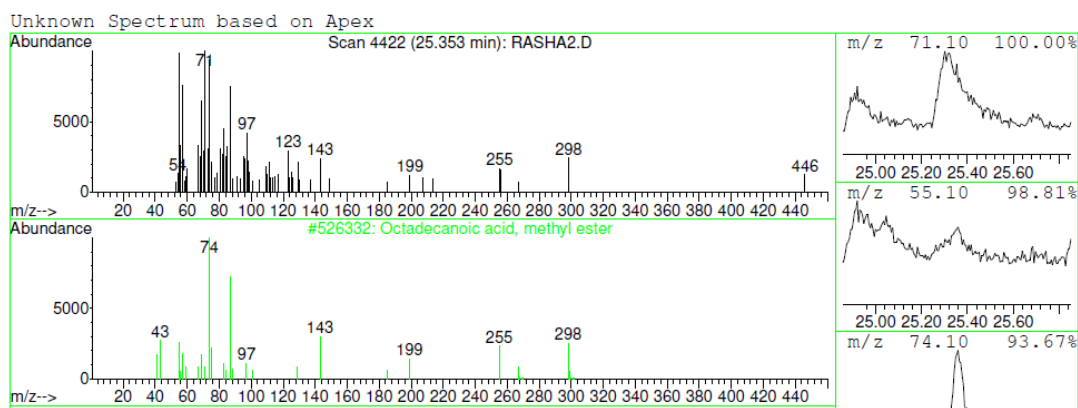


Figure (8): Mass fragment of octadecanoic acid methyl ester (methyl stearate)

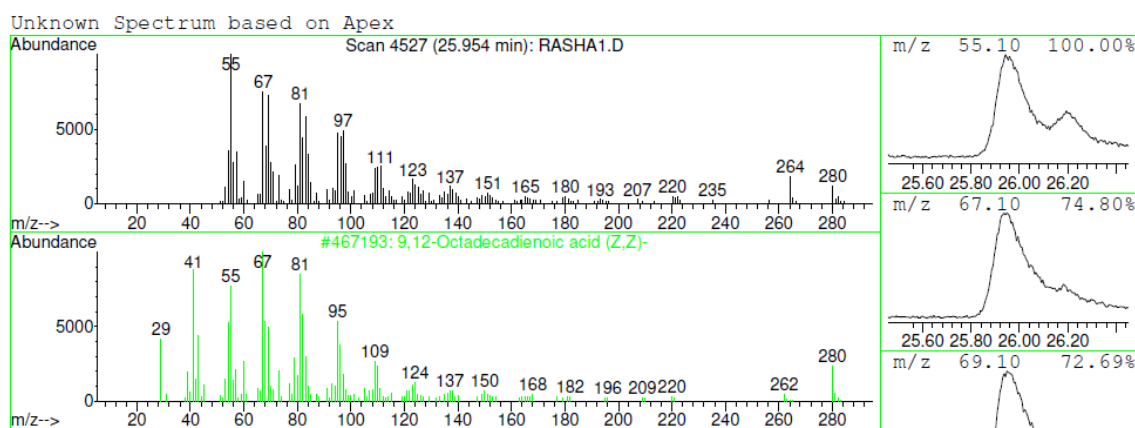


Figure (9): Mass fragment of linoleic acid (omega 6)

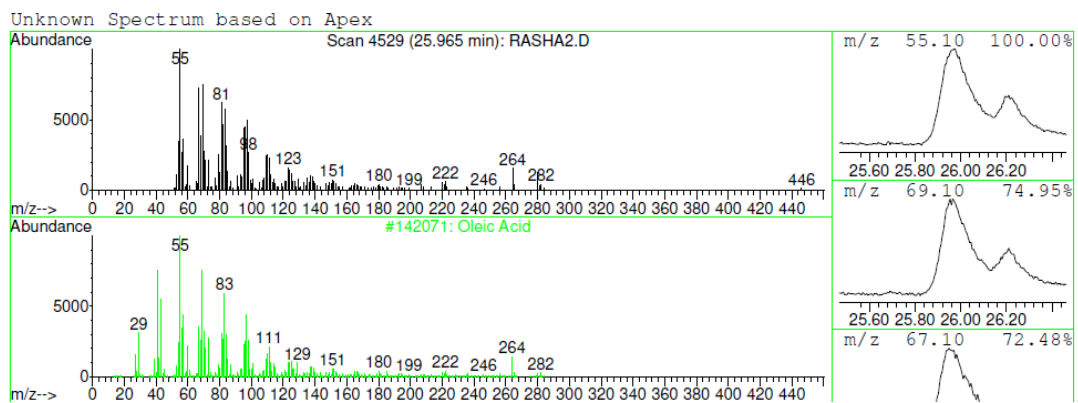


Figure (10): Mass fragment of oleic acid (omega 9)

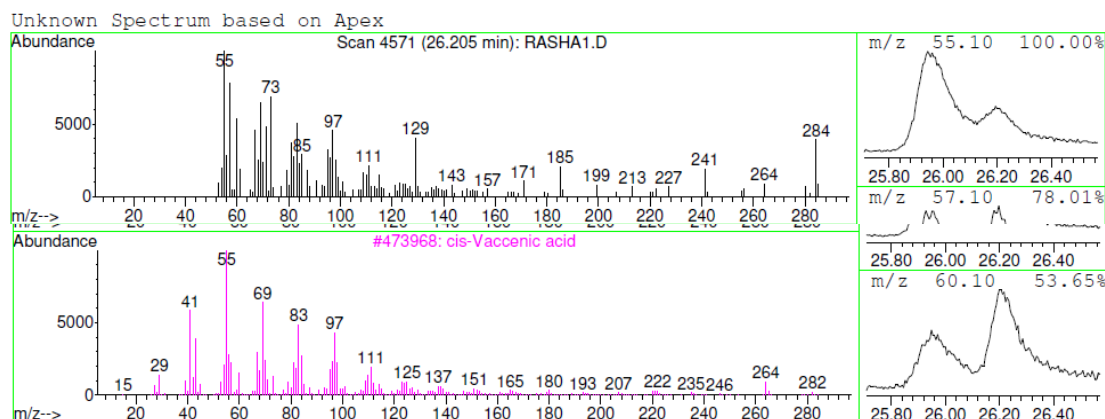


Figure (11): Mass fragment of omega7 (cis-vaccenic acid)

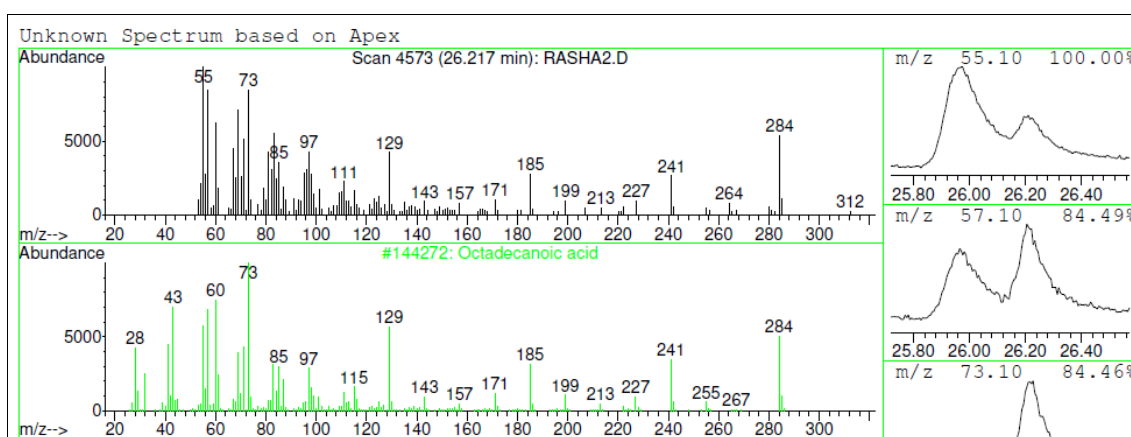


Figure (12): Mass fragment of octadecanoic acid (stearic acid)

A comparable results were detected from the seeds extract of the plant collected from Egypt [12], India [13], Jordan [14], Romania [15], and Iran [16] in which stearic acid, palmitic acid, linoleic acid, linolenic and oleic acid were detected in the seeds of the plant in different concentrations while arachidic and behenic acid were reported in the Indian, Romanian, and Jordanian seeds but not detected in the Egyptian, Iranian and Iraqi seeds.

The non-polar ingredient of the leaves and the flowers are not investigated elsewhere. Phytosterols, catechin and Monosaccharides detected by GC-MS in *Silybum marianm* leaves, flowers and seeds

To boost the sensitivity of the separation and identification process of the active compounds a derivatization reaction was done to the ethanolic extract [17] using

silylation reaction, since that the trimethylsilyl derivative posse a lower boiling point than the original one, which decrease the chance of compound decomposition when injected or at the column[18]. The result of GC-MS Fig (13, 14 and 15) show numerous monosaccharides detected in the leaves, flowers and seeds ethanolic extracts Table(2) while catechin and phytosterols were detected only in the seeds Table(3) the mass fragment of each compound are shown below in Fig (16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, and 36).

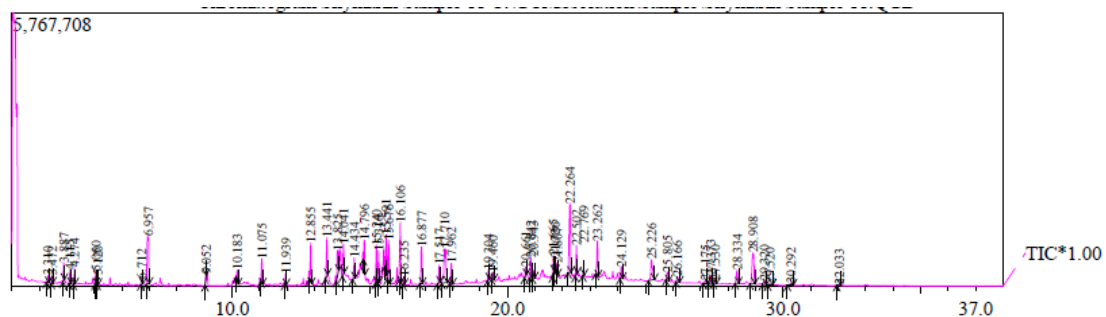


Figure (13): GC-MS chromatogram of ethanolic extract of *Silybum marianum* seeds

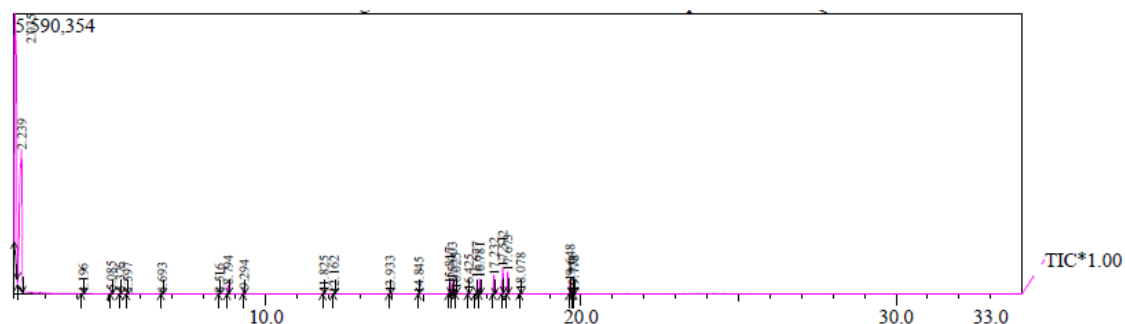


Figure (14): GC-MS chromatogram of ethanolic extract of *Silybum marianum* flowers

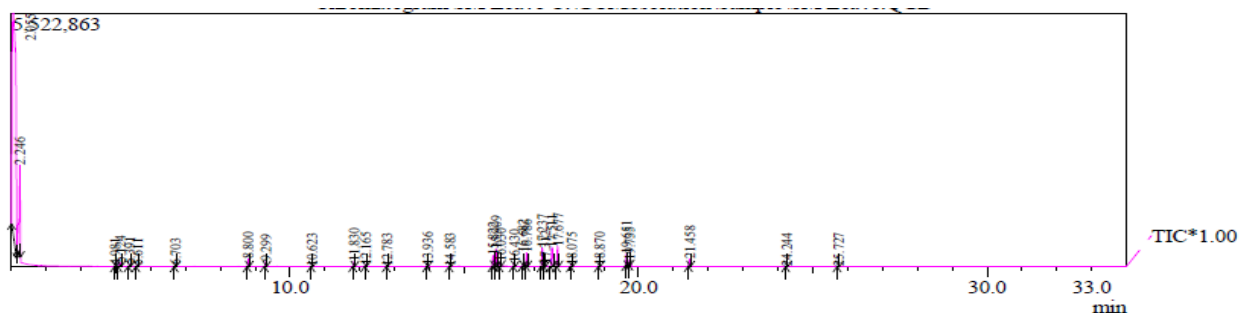


Figure (15): GC-MS chromatogram of ethanolic extract of *Silybum marianum* leaves

Table (2): -Monosaccharides content of *Silybum marianum* leaves, seeds and flowers

| Compound | Retention time | Area | | |
|--|----------------|--------|-------|---------|
| | | leaves | seeds | flowers |
| 2-Deoxy ribose O, O', O''-tris(trimethylsilyl) | 11.942 | - | 0.68% | - |
| D-Fructose, 1,3,4,5,6-pentakis-O-(trimethylsilyl)- | 14.042 | 1.52% | 0.68% | 1.21% |
| mannose | 14.8 | - | 1.82% | - |
| Sedoheptulose | 15.317 | - | 2.21% | - |
| Glucopyranose, pentakis-O-trimethylsilyl | 15.675 | 1.05% | 4.03% | 1.27% |
| 2-Deoxy-galactopyranose, tetrakis(trimethylsilyl) | 16.025 | - | - | 0.3% |
| Sorbopyranose, 1,2,3,4,5-pentakis-O (trimethylsilyl)- | 16.033 | 0.41% | - | - |
| Myo-Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)- | 16.233 | 2.5% | 3.85% | 2.92% |
| D-Ribose, 3-O-methyl-2,4,5-tris-O-(trimethylsilyl)-, | 16.433 | 0.31% | - | 0.23% |
| Trimethylsilyl ether of glucitol | 17.233 | 1.67% | - | 1.63% |
| d-Galactose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-, o-methyloxyme, (1Z)- | 17.308 | 0.19% | - | - |
| D-Glucose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)- | 17.675 | 1.68% | - | 1.9% |
| Maltose, octakis(trimethylsilyl)- | 19.650 | 1.12% | - | 1.18% |
| Mannose, 6-deoxy-2,3,4,5-tetrakis-O-(trimethylsilyl)-, | 20.842 | - | 1.5% | - |
| alpha. -D-Glucopyranoside, 1,3,4,6-tetrakis-O-(trimethylsilyl)-. | 21.725 | - | 3.22% | - |
| beta. -L-Galactopyranose, 6-deoxy-1,2,3,4-tetrakis-O-(trimethylsilyl)- | 23.258 | - | 3.26% | - |
| Per-O-trimethylsilyl-(3-O-. beta. -d-mannopyranosyl-d-glucitol) | 29.317 | - | 5.66% | - |

Table (3): phytosterols and Catechin content of *Silybum marianum* seeds

| Compound | Retention time | area |
|-------------------------------|----------------|-------|
| Catechin | 24.125 | 1.12% |
| Beta. -Sitosterol | 27.175 | 0.26% |
| Stigmasterol | 27.533 | 0.38% |
| Ethyl-iso-allylcholate | 30.292 | 0.38% |

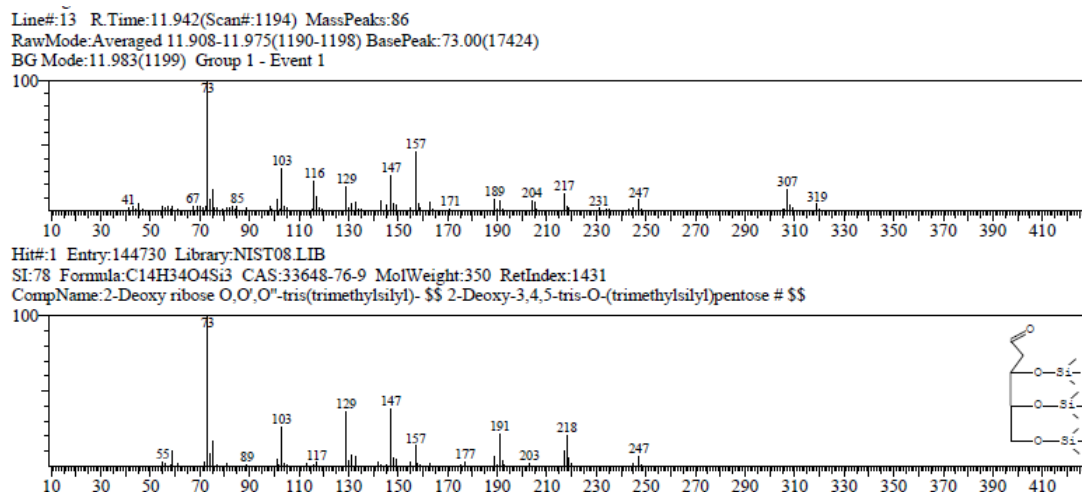


Figure (16): Mass fragment of 2-deoxy ribose

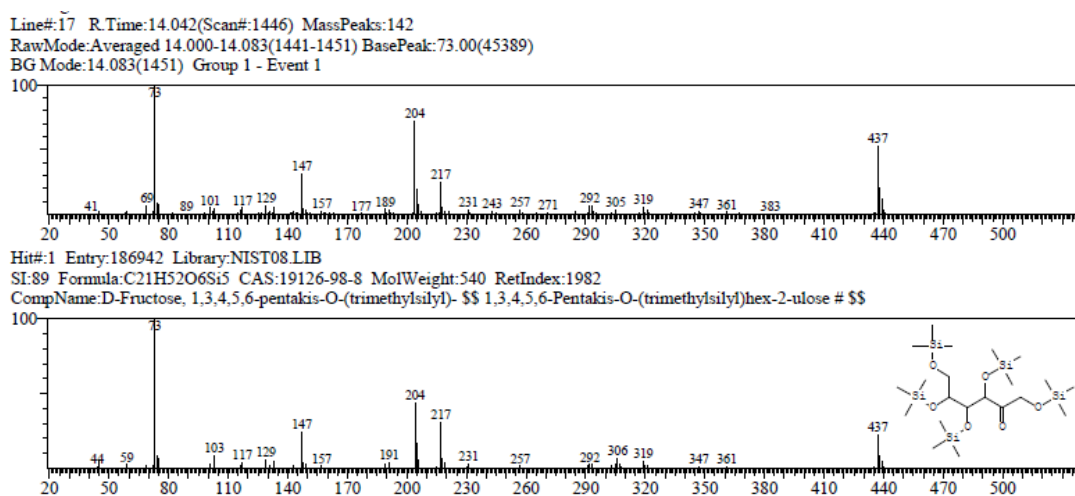


Figure (17): Mass fragment of D-fructose

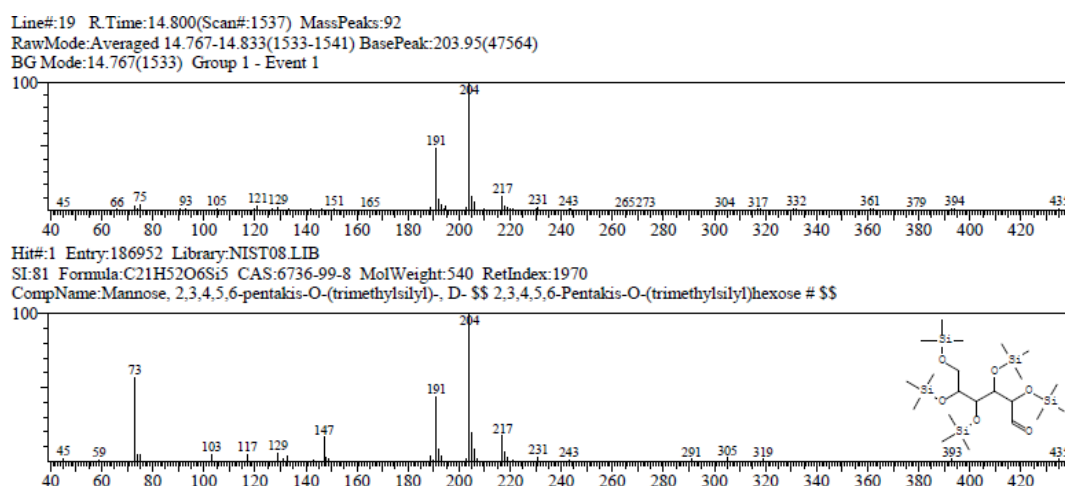


Figure (18): Mass fragment of mannose,2,3,4,5,6-pentakis-o-(trimethylsilyl)

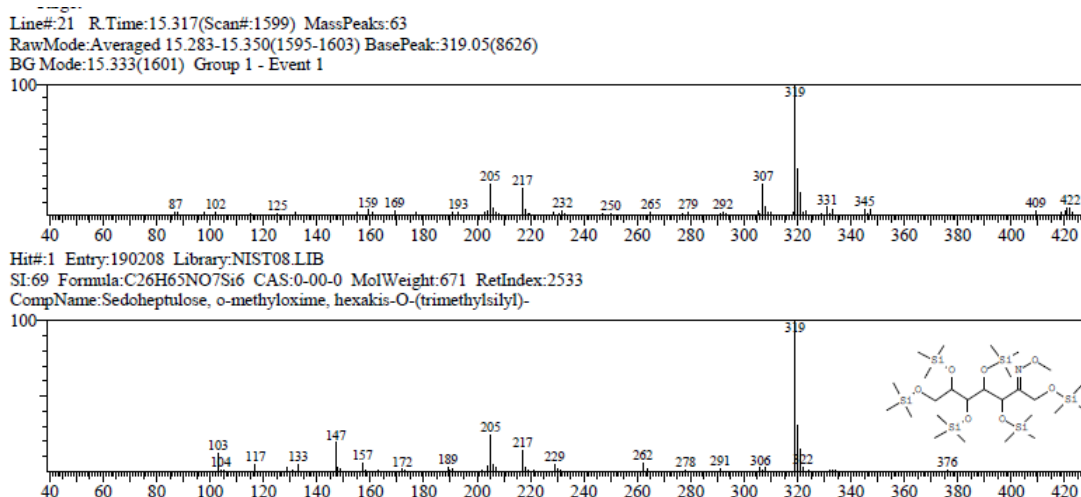


Figure (19):Mass fragment of sedoheptulose

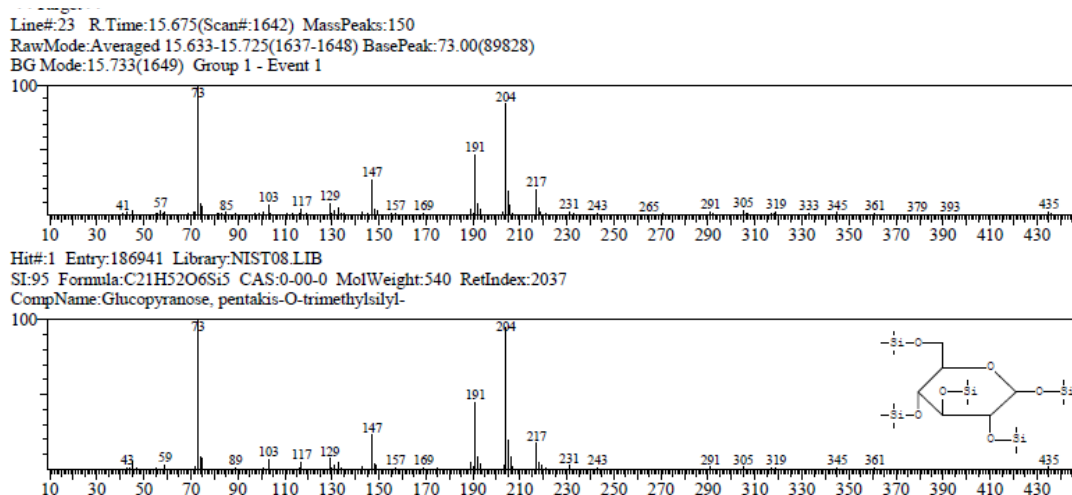


Figure (20): Mass fragment of glucopyranose

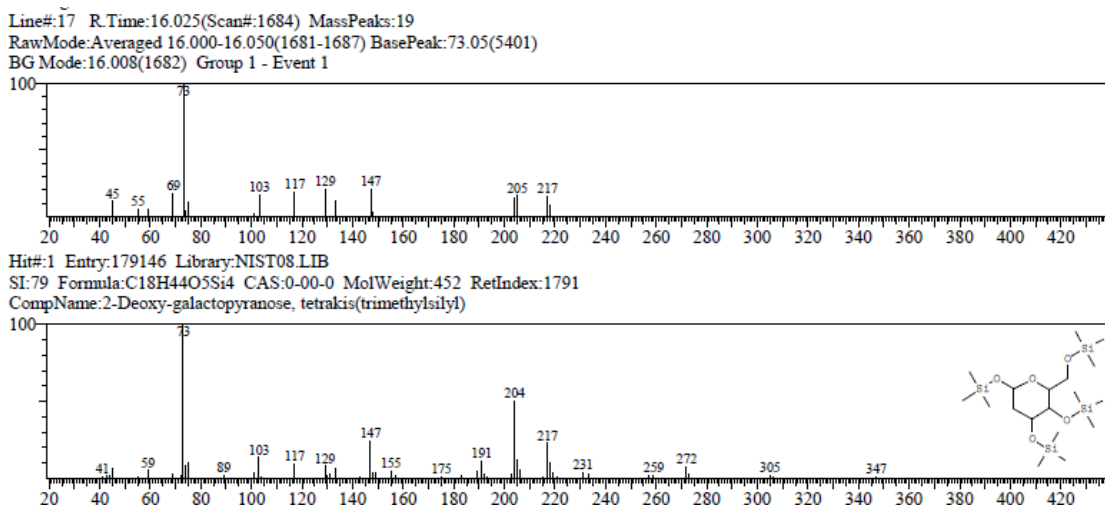


Figure (21): Mass fragment of 2-deoxy-galactopyranose

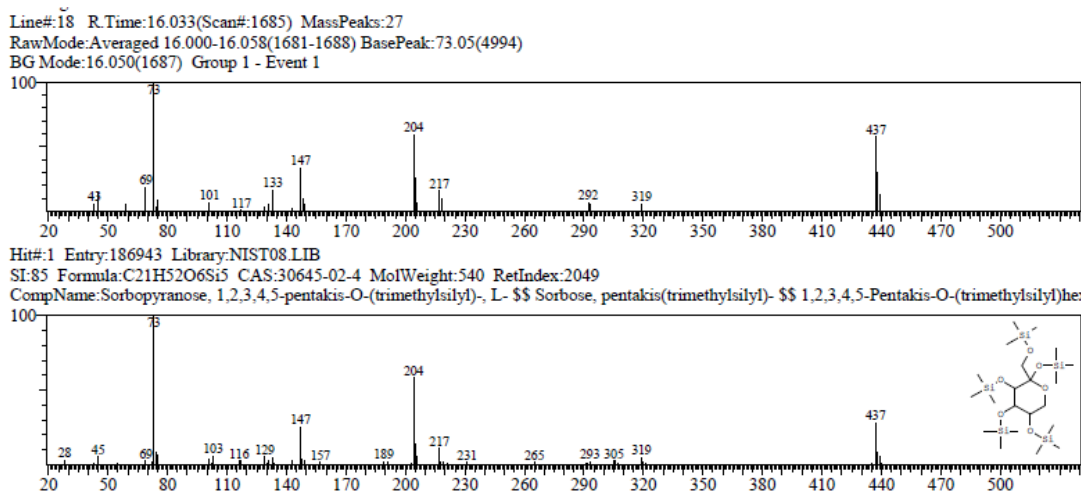


Figure (22): Mass fragment of sorbopyranose

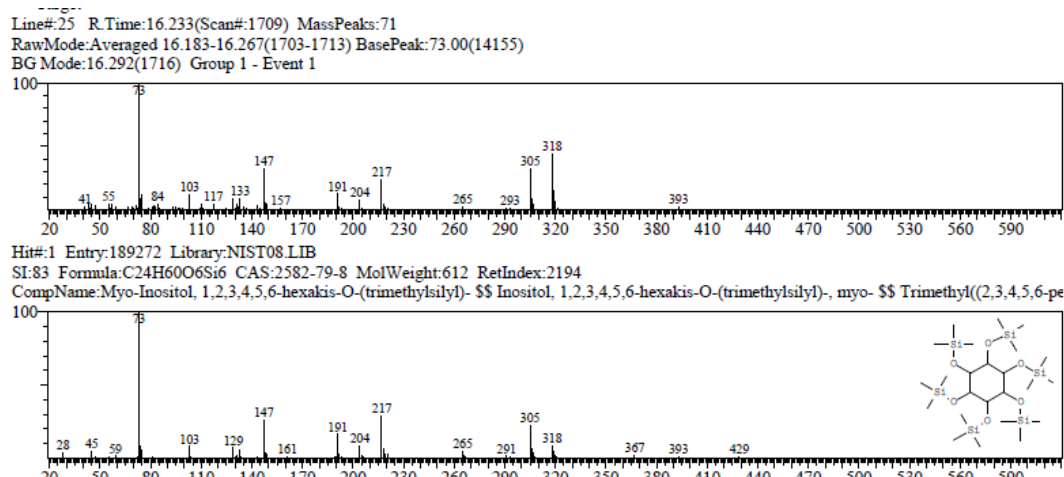


Figure (23): Mass fragment of myo-inositol

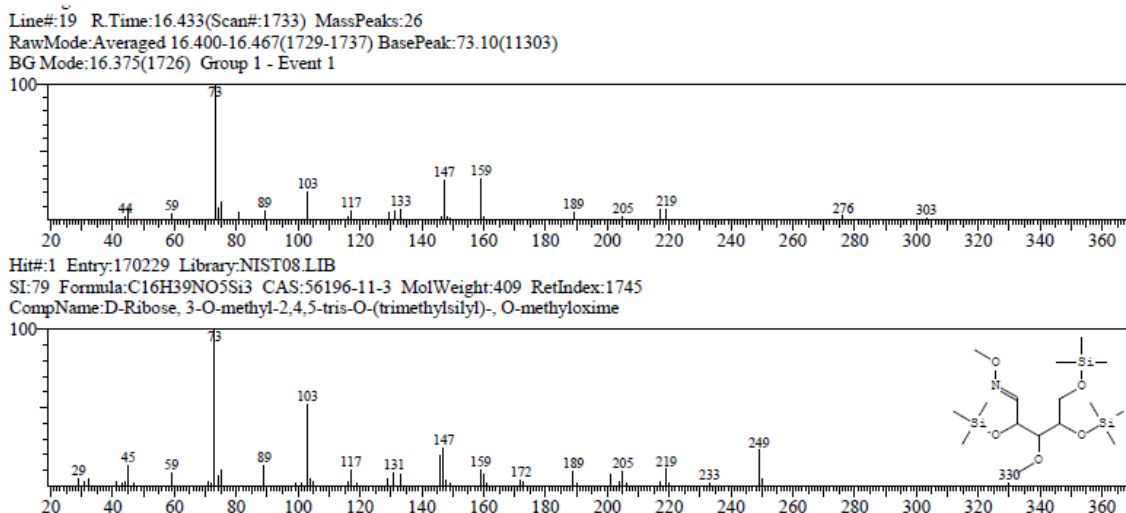


Figure (24): Mass fragment of D-ribose

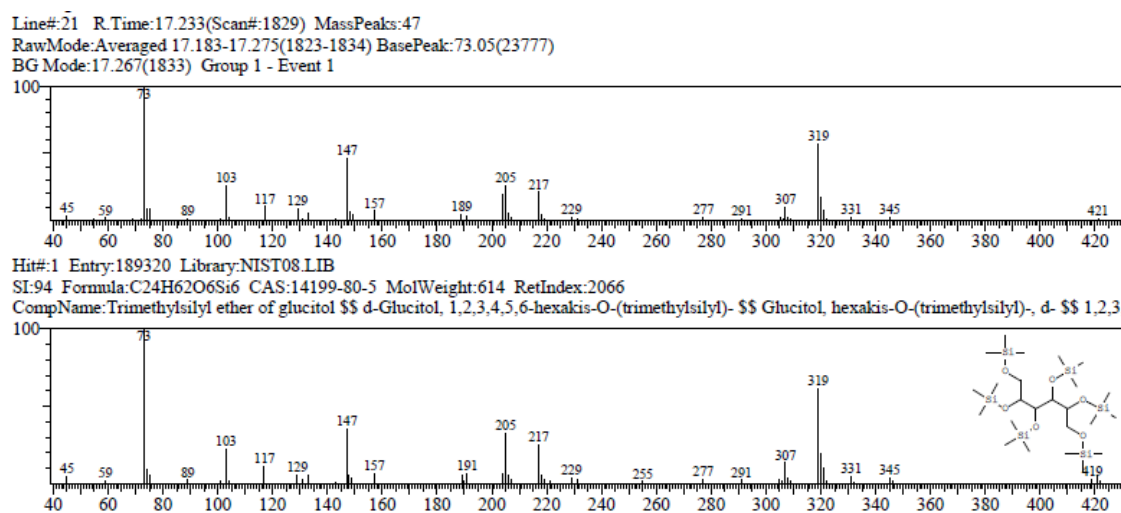


Figure (25): Mass fragment of glucitol

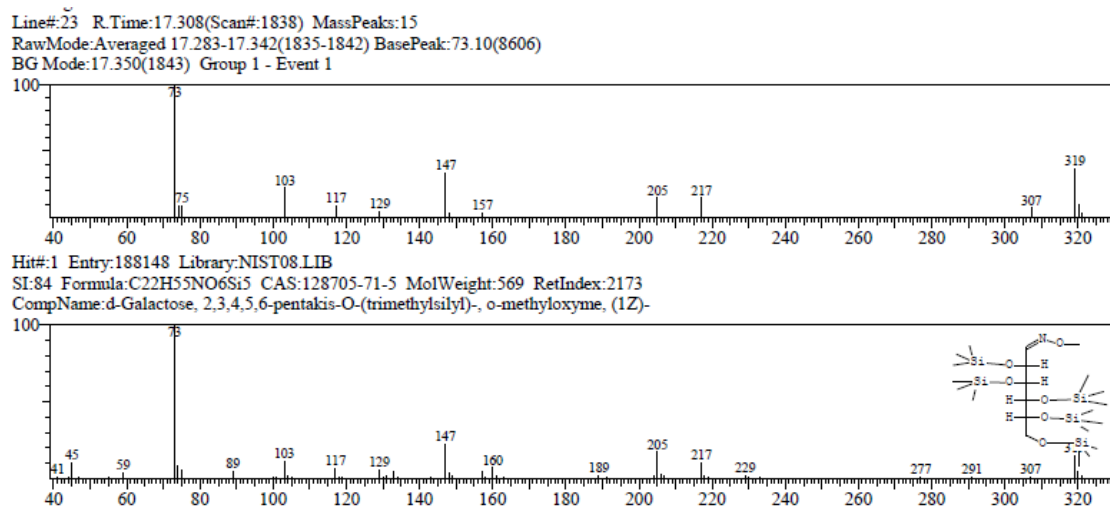


Figure (26): Mass fragment of D- galactose

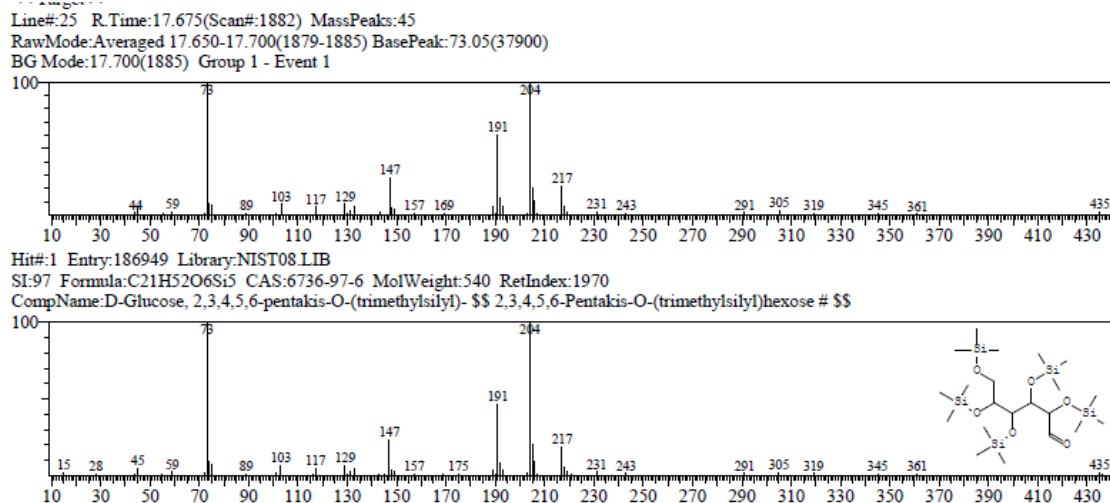


Figure (27): Mass fragment of D- glucose

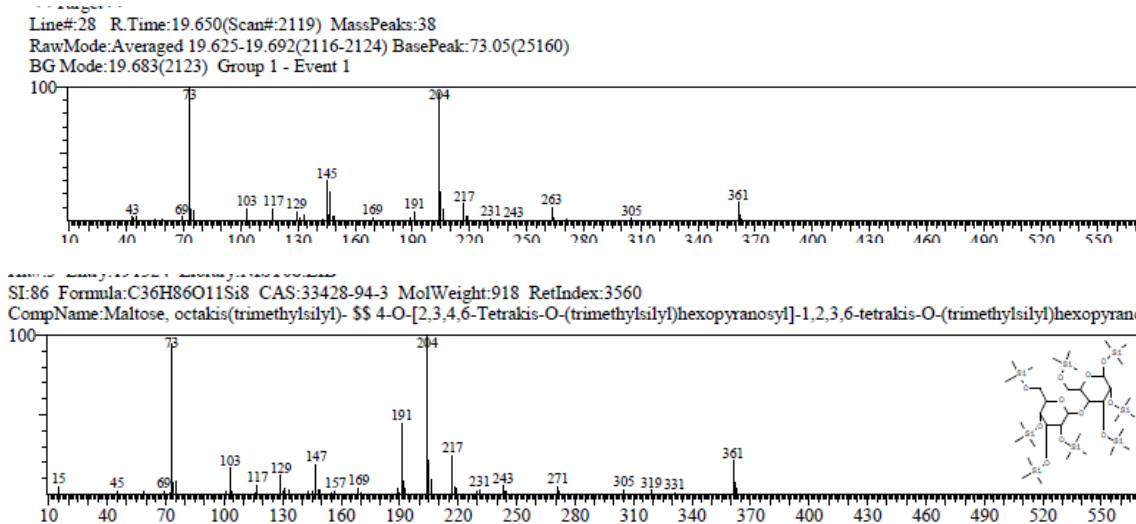


Figure (28): Mass fragment of maltose

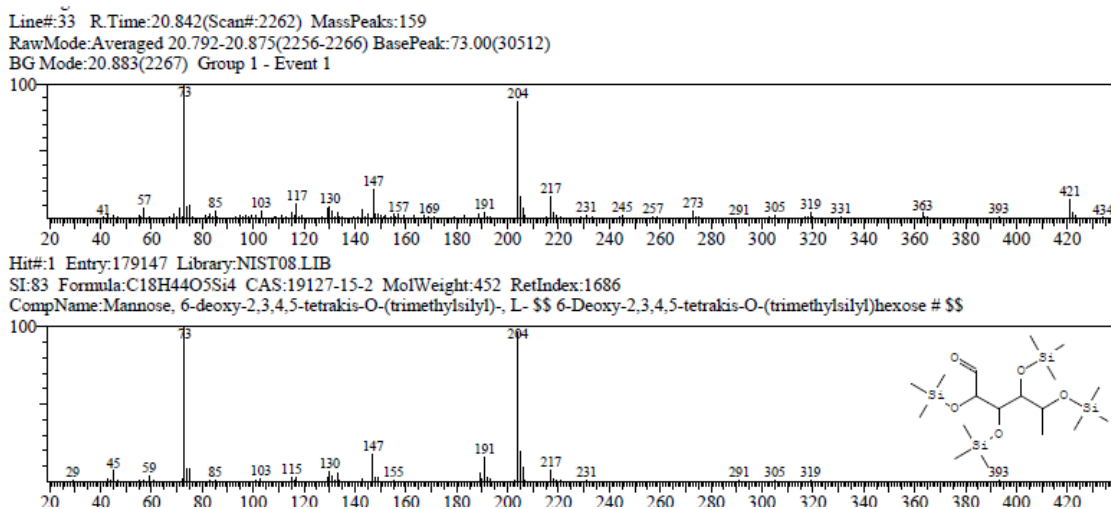


Figure (29): Mass fragment of mannose, 6-deoxy

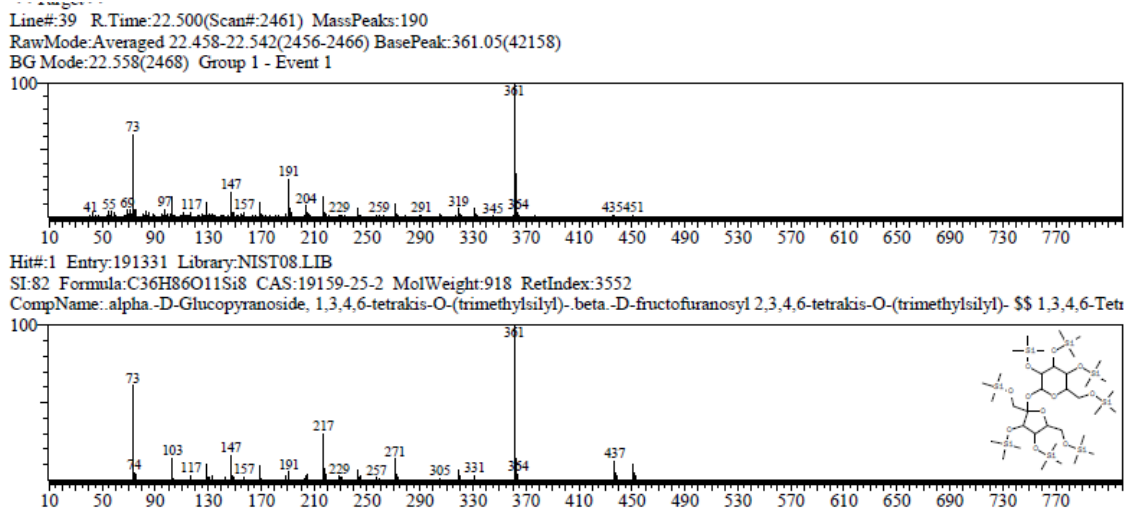


Figure (30): Mass fragment of alpha- D- glucopyranoside

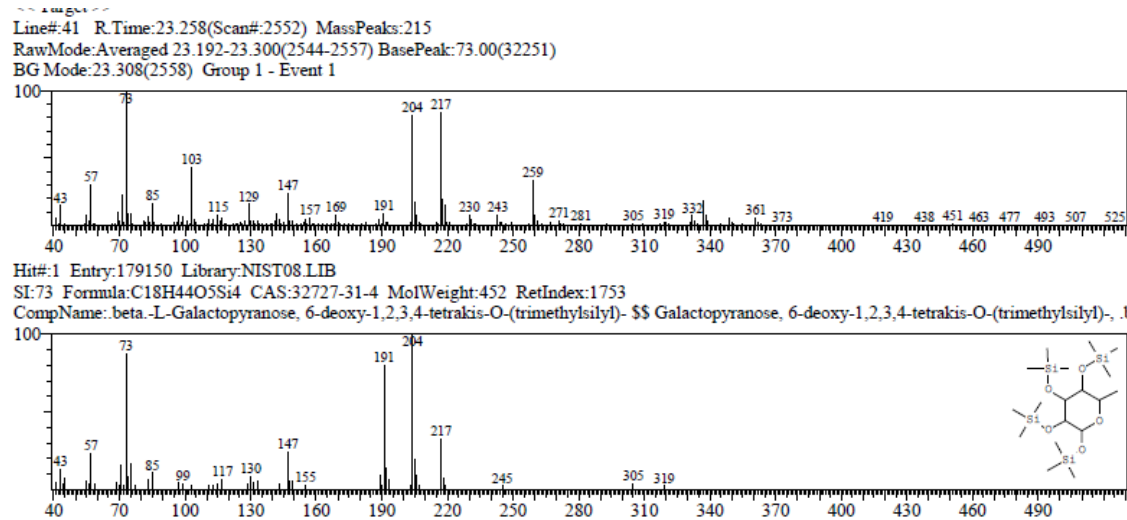


Figure (31): Mass fragment of beta-L- galactopyranose

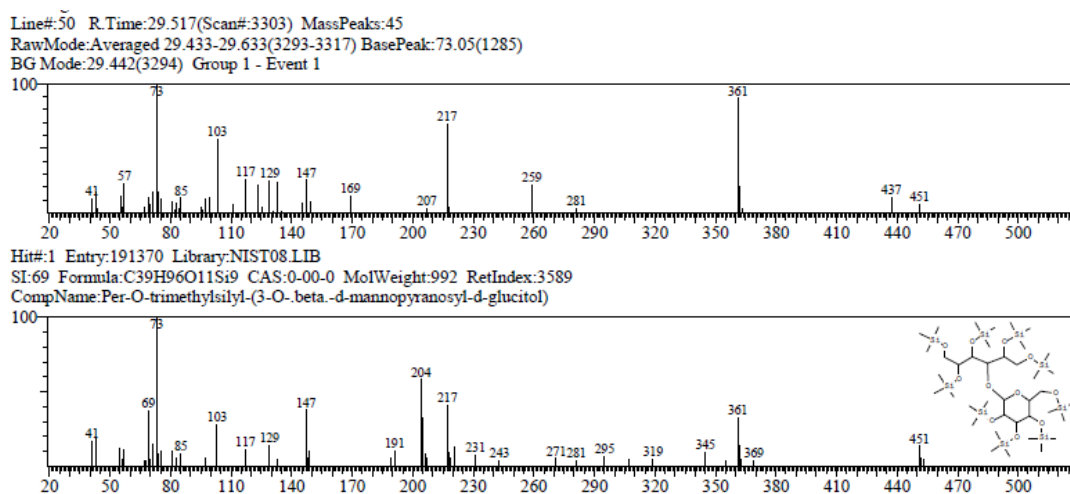


Figure (32): Mass fragment of 3-O-beta-d-mannopyranosyl-d-glucitol

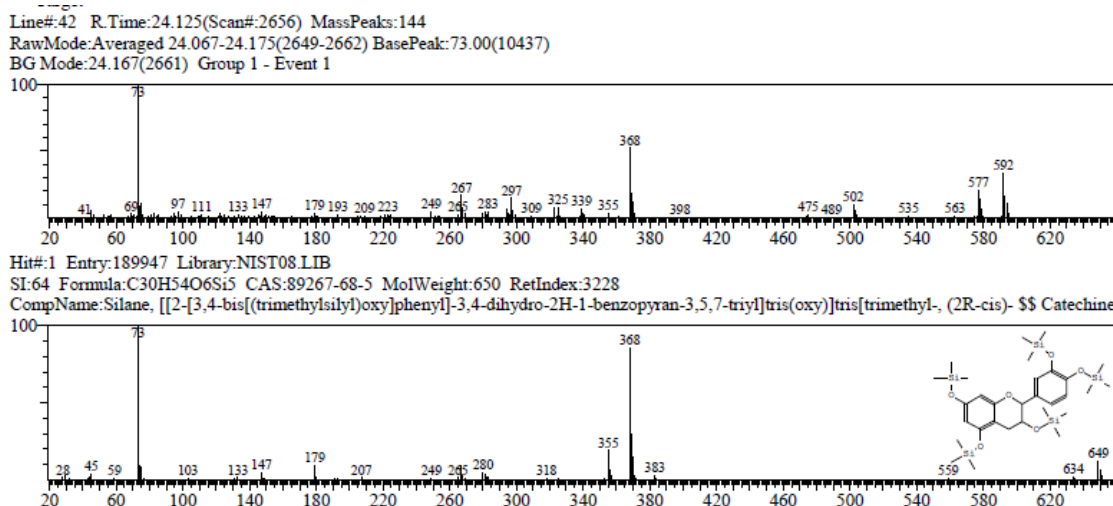


Figure (33): Mass fragment of catechin

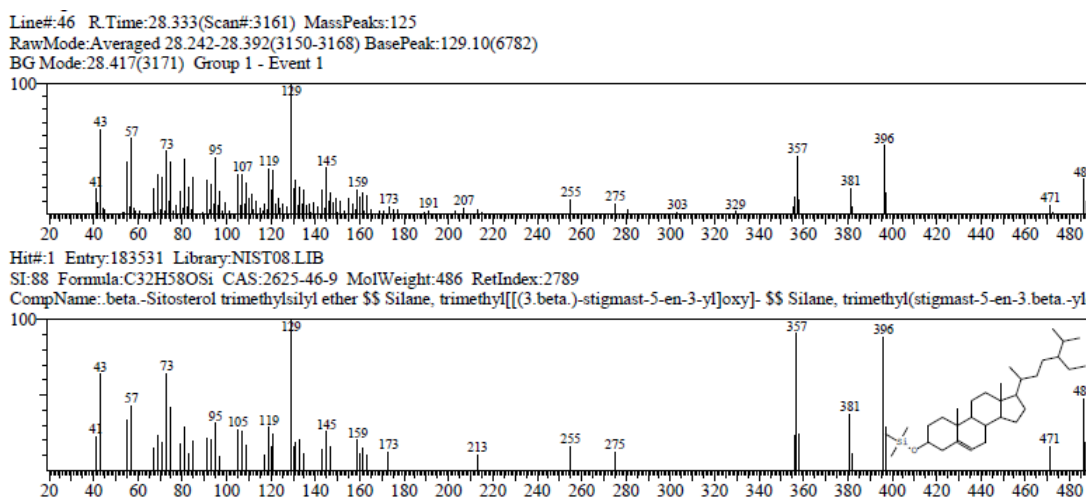


Figure (34): Mass fragment of beta-sitosterol

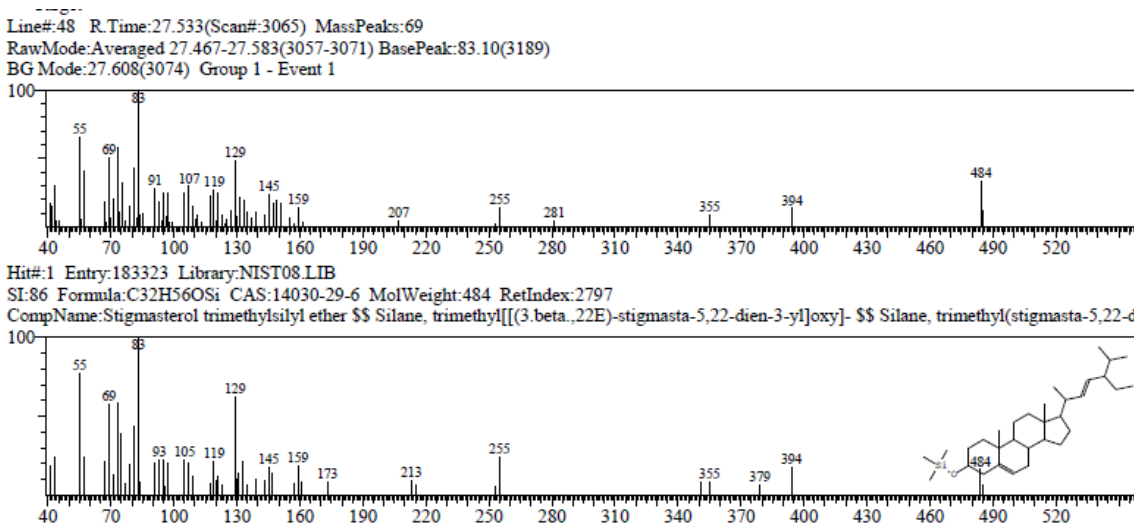


Figure (35): Mass fragment of stigmasterol

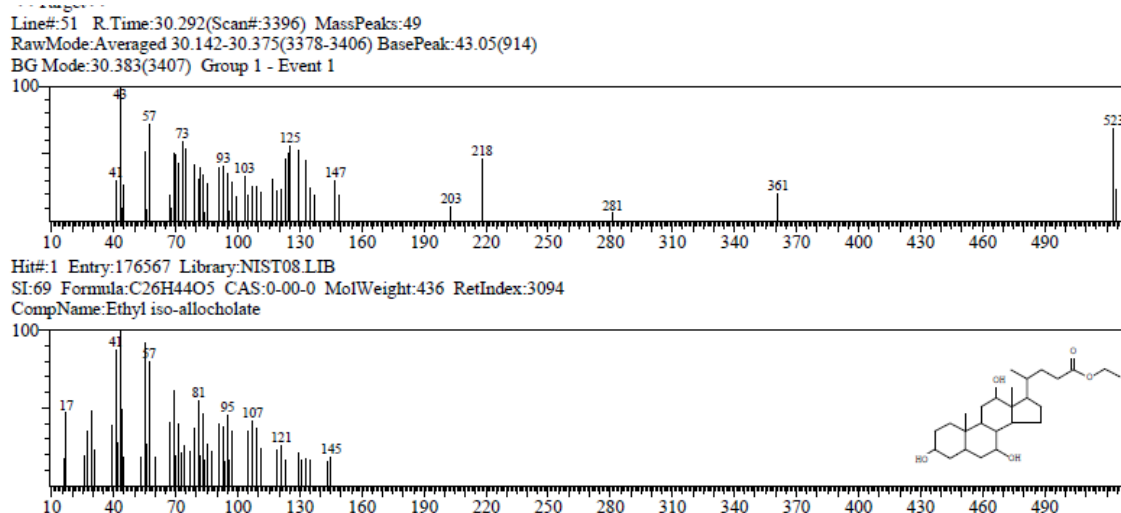


Figure (36): Mass fragment of ethyl-iso- allocholate

Only a few studies have demonstrated carbohydrate component of *S. marianum* that Yao et. al [19] and Zhaunbaeva et. al[20]. have detected glucose, galactose and mannose in the aerial part of *S. marianum* grown in China and Uzbekistan, which also contain rhamnose, xylose and arabinose that not detected in Iraqi plant, while Yasin et. al. and Nian et. al [21] have also reported mannitol, myo-inositol and galactose in the stem of Iraqi plant, which also contain sucrose, fructose, raffinose and arabinose that are not detected in the seeds, flowers and leaves, while our study have detected fructose, glucopyranose, ribose, sedoheptulose, galactopyranose, sorbopyranose, glucitol and maltose that are not reported elsewhere.

Stigmasterol and beta-sitosterol have been detected in the seed of *Silybum marianum* grown in different countries such as Egypt [22], Bulgaria [23], Poland [24], Morocco [25], and Tunis [26], Catechin and ethyl-iso- allocholate is detected for the first time in *S.marianum* plant.

Conclusion: -

A-From GC-MS result of *S. marianum* hot hexane extracts of flowers, leaves and seeds we can conclude the following points: -

1-Unsaturated fatty acids were of the higher concentrations in all parts, that oleic acid(omega9) was the predominant in the

leaves (35.15%) and seeds (33.7%) and present only by (0.87%) in the flowers, which contain glucopyranose and myo-inositol in linoleic acid (omega 6) in higher concentration (33.3%) and it have been detected in the seeds by (9.88%) and in leave by small percent (1.99%), while Cis-vaccenic acid (omega 7) has only detected in the flowers by (15.5%).

2-Saturated acids are detected in the flowers and leaves but not in the seeds while their ester have been detected in all parts; palmitic acid was the predominantly the higher in flowers (29.26%) followed by leaves (25.6%) and not detected in the seeds; stearic acid was detected only in the leaves by (15.45%); methyl palmitate and methyl stearate are detected in all plant parts in small percent.

3-Fatty alcohol are detected in small percent; palmityl alcohol (3.29%) and (1.25%) in the leaves and flowers respectively while behenyl alcohol was detected in the leaves only by (4.35%).

4-Volatile oil such as phytol, which is diterpene (C20) present in the leaves only.

B-From GC -MS result of *S. marianum* hot ethanol extract of flowers, leaves and seeds we can conclude the following points: -

1-Flowers, Leaves and Seeds of the plant contain fructose, glucopyranose and myo-inositol by different concentrations.

2-Seeds contain a no. of monosaccharides that are not present in other parts which

are: ribose, mannose, sedoheptulose, mannose-6-deoxy, alpha-D-glucopyranoside, beta-L-galactopyranose-6-deoxy and 3-O-beta-d-mannopyranosyl-d-glucitol.

3-Leaves and flowers contain a comparable ingredient where both contain D-ribose-3-O-methyl, glucitol, D-glucose and maltose does not present in the seeds.

4-Sorbopyranose and D- galactose were present only in the leaves while 2-deoxy-galactopyranose was present only in the flowers.

5-Beta-sitosterol, stigmasterol, ethyl-iso-allochololate and catechin have been detected only in the seeds of the plant.

Recommendation

Further study is required to investigate other ingredient present in the plant, which could not be detected by GC-MS such as flavonoids, alkaloids, lignans and flavolignan

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