

# Phytochemical Characterization using GC-MS Analysis of Methanolic Extract of *Moringa oleifera* ( Family Moringaceae) Plant Cultivated in Iraq

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## Abstract

**Objective:** The aim of the present study was to characterize the *Moringa oleifera* plant cultivated in Iraq for the presence of biologically active phyto-chemicals using methanolic extracts of the plant (leaves and seeds) . This study was determined by using Gas Chromatography –Mass spectrometry, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library.

**Methods:** In the present investigation, methanolic extracts of *Moringa oleifera* were screened for the presence of steroids, alkaloids, tannins, flavonoids, terpenoids, anthraquinoin and cardiac glycosides by standard qualitative test procedures and further this study was extended by analyzing the potent bioactive compounds in the methanolic extract of plant using GC-MS analysis.

**Results:** reveal the presence of different compounds (about 100 compound) in the *Moringa* plant extract among them alkaloids, terpenoids steroids, saturated and unsaturated fatty acid, aromatic and aliphatic hydrocarbon, polyphenolic compounds . GC-MS was done using the database of National Institute of standard and Technology (NIST).

**Conclusion:** Results confirmed the presence of therapeutically potent compounds in the *Moringa* extract predominantly steroids, flavonoids and terpenoids.

**Keywords:** *Moringa oleifera*, biologically active, Phytochemicals, GC-MS analysis.

## Introduction

*Moringa oleifera* Lam. (Moringaceae) , commonly known as drumstick or horseradish, It is a small, fast, growing, evergreen, or deciduous tree that usually grows up to 10 or 12 m in height , native to the Sub-Himalaya tracts of India, Pakistan, Bangladesh, Central America, Afghanistan, and Africa (Fahey JW 2005 & Anwar *et al* 2007). *Moringa*, which is rich in vegetable oil and high in nutritional values, is used in Asia as a vegetable and medicinal plant. This is attributed to the presence of proteins, vitamins, and various phenolic compounds in the oil.( Anwar *et al* 2007) . Nevertheless, all parts of the *Moringa* tree are edible and have been consumed for many years by humans. The diverse range of medicinal uses for *Moringa oleifera*, include its use as an antioxidant(Verma AR. *et al* 2009), anti-carcinogenic(Bharali R *et al* 2003), anti-inflammatory, antispasmodic, diuretic(Cáceres A *et al* 1992), antiulcer, antibacterial, antifungal(Caceres A *et al* 1991 ) and its antinociceptive(Sulaiman MR *et al* 2008 ) properties, as well as its wound healing ability has been demonstrated(. Rathi BS *et al* 2006). Additionally, the root bark has been used as an analgesic, alexeteric, anthelmintic, and treatment for heart complaints, as well as for eye diseases, inflammation and dyspepsia.( Nadkarni KM 1976 & Chopra R *et al* 1982). Phytochemical screening is of paramount importance in identifying new source of therapeutically and industrially valuable compound having medicinal significance, to make the best and judicious use of available natural wealth. Hence, the present investigation was carried out to determine the possible phytochemical components from a new cultivated plant in Iraq (figure 1,2) by GC-MS analysis. In recent years, interest for the characterization of organic compounds from plants has been developed. Therefore, an attempt was made to screen and isolate the bioactive compounds, evaluate the bioactive potential and characterize them by GC-MS analysis

## Materials and Method

### Plant Material

The plant samples were cultivated and collected from Baghdad in Iraq , and authenticated by the National Iraqi Herbarium, Botany Directorate at Abu-Ghreib, they were dried in shade for several days at room temperature and then grinded as powder.

**The experimental work** is divided into:

- The experimental preliminary phytochemical screening of various secondary metabolites like alkaloids, flavonoids, steroids, tannins, saponins, anthraquinoin, terpenoids and cardiac glycosides) in the *Moringa* plant.
- Extraction of different active constituents.

- GC-MS analysis of methanolic extract of the plant.

#### **Preliminary qualitative phytochemical analysis:**

Chemical tests were carried out using the methanolic extracts from plants and or the powdered specimens, using standard procedures to identify the active constituents.( Kokate C *et al* 2009, Harborne J.B 1973& Sarker S. D *et al* 2005 )

#### **Test for alkaloids**

Alcoholic extract (10 ml) was stirred with 5 ml of 1% HCL on a steam bath. Mayer's(1.35gm mercuric chloride in 60ml water + 5gm potassium iodide in 10ml water )and Wagner's reagents (1.27g of iodine and 2g of potassium iodide in 100ml of water) were added, white and reddish brown color precipitate respectively, were taken as evidence for the presence of alkaloids.

#### **Test for flavonoids**

(i)Lead acetate test: Lead acetate 10% (1 ml) solution was added to 5ml of alcoholic extract, The formation of a yellowish- white precipitate was taken as a positive test for flavonoids.

(ii)NaOH test: The extract (5 ml) was treated with aqueous NaOH and HCl, and looking for the formation of a yellow orange color.

#### **Tests for steroids**

(i) Liebermann-Burchard test: Extract (3ml) was treated with chloroform, acetic anhydride and drops of sulphuric acid was added. The formation of dark pink or red color indicates the presence of steroids.

(ii)H<sub>2</sub>SO<sub>4</sub> test: The development of a greenish color was considered as indication for the presence of steroids, when the organic extract (2 ml) was treated with sulphuric and acetic acids.

#### **Test for tannins**

Plant material (10mg) in 10ml distilled water was filtered, then the filtrate (3ml) + 3ml of FeCl<sub>3</sub> solution (5%w/v) were mixed. The formation of a dark green or blue black precipitate was considered an indication for the presence of tannins.

#### **Tests for anthraquinones**

Borntrager's test: 3ml of alcoholic extract was shaken with 3 ml of benzene, filtered and 5 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the development of a pink, red or violet color in the ammonical (lower) phase indicates the presence of free anthraquinones.

#### **Test for terpenoids**

Alcoholic extract (2ml) was dissolved in chloroform (2ml) and evaporated to dryness. concentrated sulphuric acid (2ml) was then added and heated for about 2 min. A grayish color was considered an indication for the presence of terpenoids.

#### **Test for cardiac glycoside**

Keller-kiliani test: Alcoholic extract (2ml) +1ml glacial acetic acid+ FeCl<sub>3</sub>+con.H<sub>2</sub>SO<sub>4</sub>. Formation of green-blue color indicates the presence of cardiac glycoside.

#### **Preparation of extract**

Shade-dried coarsely powdered seeds (120) separately were defatted with hexane for 24 hours then allowed to dry at room temperature. The defatted plant materials was extracted with 80% methanol in soxhlet apparatus until complete exhaustion . The alcoholic extract was evaporated under reduced pressure at a temperature not exceeding 40 C to give a dark-brown residue designated as a crude extract . The alcohol extract then was subjected to GC-MS analysis.

#### **GC-MS analysis**

##### **Instruments and chromatographic conditions**

GC-MS analysis was carried out on GC-MS-QP2010 Shimadzu system comprising a gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions : column VF-5MS fused silica capillary column (30.0m x 0.25mm x 0.25µm, composed of 5% phenyl/95% dimethylpolysiloxane), operating in electron impact mode at 70ev; helium (99.999%) was used as carrier gas at a constant flow of 1. ml/min and an injection volume of 0.5µl was employed (split ratio of 10:1) injector temperature 240 0C ion-source temperature 200 0C. The oven temperature was programmed from 70 0C (isothermal for 3 min), with an increase of 10 0C/min, to 240 0C, ending with a 9min isothermal at 280 0C. Mass spectra were taken at 70ev; a scan interval of 0.5 seconds and fragments from 40 to 440Da. Total GC running time is 40min.



Figure1- *Moringa oleifera* cultivated in Iraq

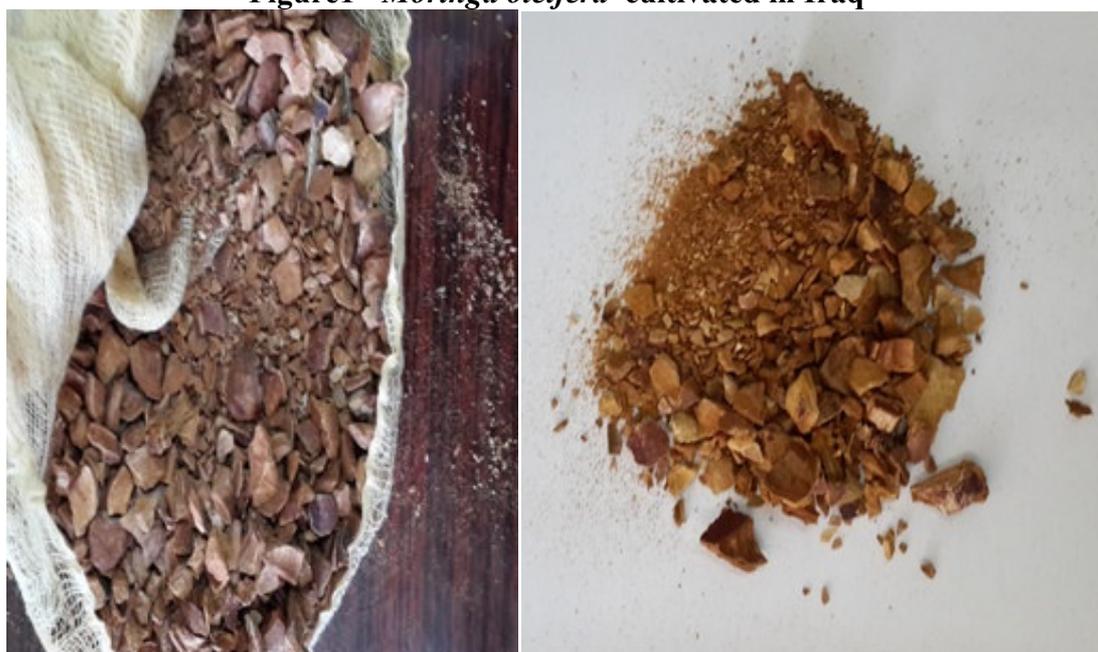


Figure2- *Moringa oleifera* seeds

## Results

The results of preliminary qualitative phytochemical analysis are given in (table-1)

**Table-1: Phytochemical screening of *Moringa oleifera* extract**

| Alkaloids | Flavonoids | Steroids | Tannins | Saponins | Anthraquinoin | Terpenoids | Cardiac glycoside |
|-----------|------------|----------|---------|----------|---------------|------------|-------------------|
| +         | +          | +        | +       | -        | -             | +          | +                 |

+, - represent presence and absence of phytoconstituents respectively.

The results of preliminary phytochemical screening of plant extracts showed the presence of alkaloids, flavonoids, steroids, tannins and terpenoids and cardiac glycoside in the Iraqi Moringa, and the absence of

saponins and anthraquinoin in this plant . Many researchers reported that the concentration of secondary metabolites are varying from plant to plant belong to the same genus and even in the different parts of the same plant(Abdul K. *et al* 2009) , this is due to many factors like environmental heterogeneity, since the effect of environmental heterogeneity is highly scale-dependent. It may create high niche diversity and hence allow species to coexist at a large spatial scale(Pausas J. &, Austin M 2001), also the high complexity and heterogeneity of soil, like( soil structure, texture and depth, moisture retention characteristics, aeration) create a big variation in the chemical constituents even in the same country (Karlovsky P 2008).

#### Identification of Components by GC-MS:

Interpretation on mass spectrum of GC-MS was done using the database of National Institute of standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library . The name, molecular weight and mass fragmentation of some of the components of the test materials were ascertained

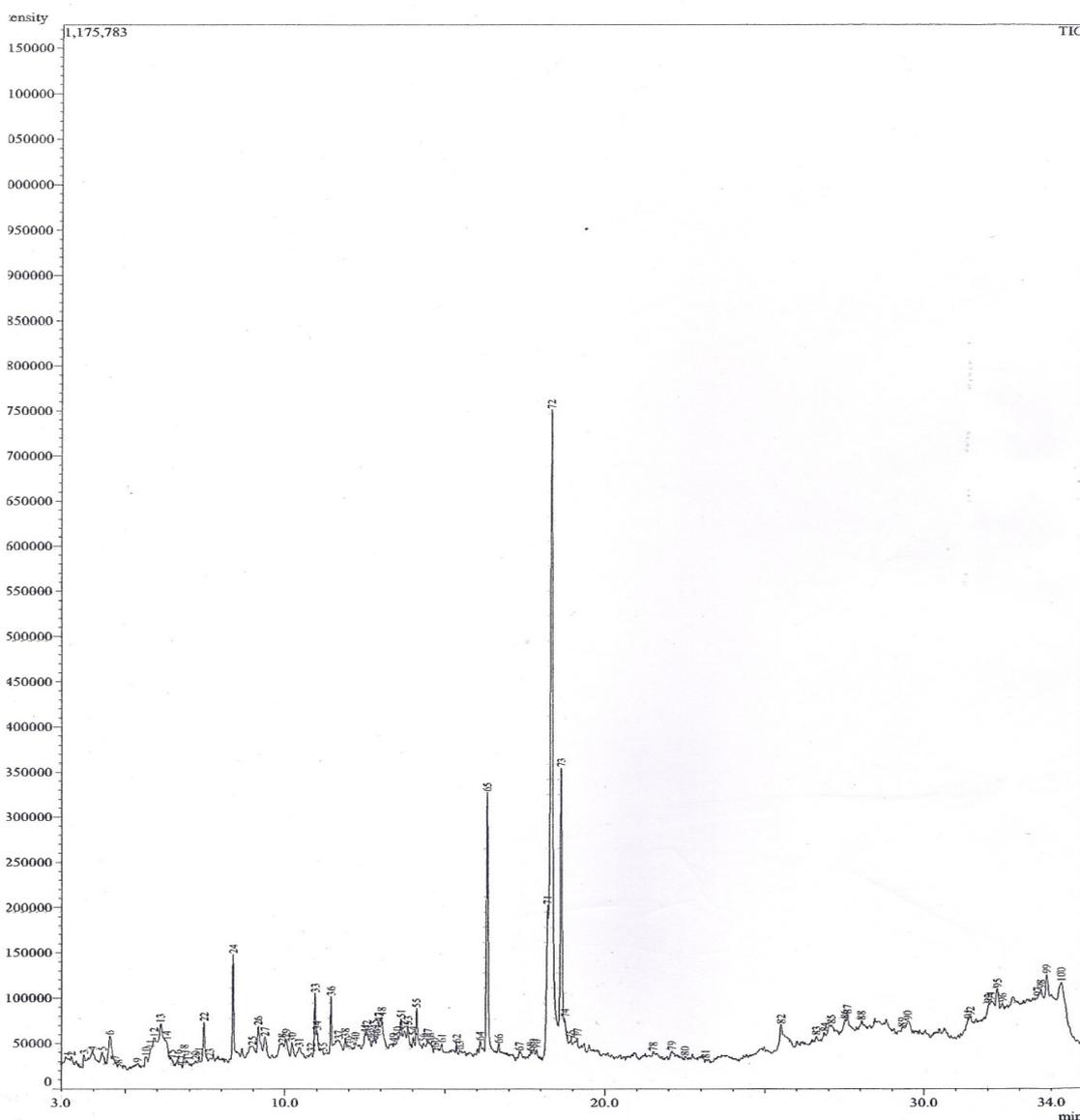


Fig3- GC-MS Chromatogram of methanolic extract of *Moringa oleifera*

The results pertaining to GC-MS analysis led to the identification of number of compounds from the methanol extract of *Moringa oleifera* plant. GC-MS chromatogram showed 100 peaks, indicating the presence of 100 compounds (fig.3) and (tab. 2).many of these components reported in this plant for the 1<sup>st</sup> time like Pyrazoline

alkaloids : { ( 2-Pyrazoline, 1-isopropyl-5-methyl-) } , { Pyrrolidine, 1-(1,6-dioxooctadecyl) } , Piperidin alkaloid: { (4-Ethoxy-6-piperidin-1-yl-[1,3,5]triazine-2-carboxylic acid amide) } , { ( 6,6-Dimethoxy-piperidin-2-one) } , { 4-Chloro-6-[2,2,2-trifluoroethoxy]-1,5-naphthyridine } , { Pyridin-3-ol, O-acetyl-2-[S-[2-acetoxyethyl]dithio]- } , quinoline alkaloids : { quinoline ,3-methyl } , { 1H,3H-Quinoline-2,5-dione, 1-(4-fluorophenyl) } , mono, di, tri and sesquiterpene compounds: { neocurdione } , { 2-exo-hydroxy-5-ketobornane } , { copaene } , { squalane } , {  $\beta$ -amyrene } , { lupenon } , caryophyllene } , { (-)-.alpha.-panasinsen } , polyphenolic compounds : { Phenol, 2-methoxy } , { 2-(3,7-Dimethyl-octa-2,6-dienyl)-4-methoxy-phenol } , plant sterol: { alpha-ergosterol } , { cholesterol } , { stigmasta-4,22-dien-3.beta.-ol } , { gama-sitosterol } , carboxylic acid : {  $\beta$ -methoxycinnamic acid } , { 2,5-Dihydro-2-ethyl-2,4-dimethyl-5-oxofuran-3-carboxylic acid } , polycyclic aromatic hydrocarbon like naphthalene and thiophenes compounds like Methyl 3-bromobenzo(b)thiophene-6-carboxylate

**Table-2: Phytocomponents identified in the methanolic extracts of *Moringa oleifera***

| Peak# | R.Time | Area   | Area% | Height | Height% | Name   |
|-------|--------|--------|-------|--------|---------|--|
| 1     | 3.13   | 61413  | 0.40  | 7182   | 0.24    | 2-Propenoic acid, oxiranylmethyl ester         |
| 2     | 3.32   | 28395  | 0.19  | 8703   | 0.29    | 2,2-Dimethoxybutane                            |
| 3     | 3.67   | 69445  | 0.03  | 28643  | 0.11    | 2-Pyrazoline, 1-isopropyl-5-methyl-            |
| 4     | 3.96   | 39700  | 0.26  | 9353   | 0.31    | 3-Furaldehyde                                  |
| 5     | 4.27   | 42023  | 0.28  | 11229  | 0.37    | 3-Furanmethanol                                |
| 6     | 4.51   | 243534 | 1.59  | 32717  | 1.07    | 2-Propanone, 1,3-dihydroxy-                    |
| 7     | 4.67   | 25810  | 0.17  | 6827   | 0.22    | 4-Ethoxy-6-piperidin-1-yl-[1,3,5]triazine-2-c  |
| 8     | 4.78   | 27495  | 0.18  | 3305   | 0.11    | 4-Chloro-6-[2,2,2-trifluoroethoxy]-1,5-naph    |
| 9     | 5.38   | 20621  | 0.14  | 4179   | 0.14    | Neocurdione                                    |
| 10    | 5.65   | 71373  | 0.47  | 12520  | 0.41    | Glycerin                                       |
| 11    | 5.80   | 147621 | 0.97  | 21147  | 0.69    | Diglycerol                                     |
| 12    | 5.90   | 185617 | 1.22  | 33645  | 1.11    | Ethyl 2,2-diethoxypropionate                   |
| 13    | 6.10   | 495292 | 3.24  | 48923  | 1.61    | Propane, 1,1-diethoxy-2-methyl-                |
| 14    | 6.28   | 316576 | 2.07  | 30196  | 0.99    | Glycerin                                       |
| 15    | 6.45   | 48997  | 0.32  | 9845   | 0.32    | Undecanedioic acid, 4-oxo-, dimethyl ester     |
| 16    | 6.64   | 52677  | 0.34  | 9658   | 0.32    | 2-Propanol, 1,1'-oxybis-                       |
| 17    | 6.77   | 22579  | 0.15  | 3099   | 0.10    | 2-Exo-hydroxy-5-ketobornane                    |
| 18    | 6.84   | 68872  | 0.45  | 15043  | 0.49    | Piperazine, 1,4-dimethyl-                      |
| 19    | 6.94   | 20066  | 0.13  | 6955   | 0.23    | 2-Butanol, 3,3'-oxybis-                        |
| 20    | 7.19   | 33641  | 0.22  | 5225   | 0.17    | 6,6-Dimethoxy-piperidin-2-one                  |
| 21    | 7.31   | 29422  | 0.19  | 8437   | 0.28    | 2-Furancarboxylic acid                         |
| 22    | 7.46   | 191145 | 1.25  | 45533  | 1.50    | Maltol   |
| 23    | 7.63   | 29454  | 0.19  | 4757   | 0.16    | Phenol, 2-methoxy-                             |
| 24    | 8.36   | 326518 | 2.14  | 112230 | 3.69    | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-     |
| 25    | 8.97   | 85620  | 0.56  | 8500   | 0.28    | 3,4-Dihydroxy-5-methyl-dihydrofuran-2-one      |
| 26    | 9.15   | 182766 | 1.20  | 31362  | 1.03    | 2-Furancarboxaldehyde, 5-(hydroxymethyl)-      |
| 27    | 9.36   | 116240 | 0.76  | 20397  | 0.67    | 1,2,3-Propanetriol, 1-acetate                  |
| 28    | 9.92   | 120483 | 0.79  | 13222  | 0.43    | Ethylene glycol butyl ether, trimethylsilyl et |
| 29    | 10.04  | 108808 | 0.71  | 18961  | 0.62    | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-     |
| 30    | 10.22  | 76067  | 0.50  | 16277  | 0.53    | 1-Amino-4-methylpiperazine                     |
| 31    | 10.45  | 59831  | 0.39  | 10688  | 0.35    | Butanedioic acid, 2-hydroxy-2-methyl-, (S)-    |
| 32    | 10.83  | 23909  | 0.16  | 5511   | 0.18    | Furazan-3-ol, 4-amino-                         |
| 33    | 10.94  | 205266 | 1.34  | 70219  | 2.31    | Quinoline, 3-methyl-                           |
| 34    | 11.01  | 98748  | 0.65  | 28282  | 0.93    | Quinoline, 3-methyl-                           |
| 35    | 11.20  | 44806  | 0.29  | 3335   | 0.11    | Acetic acid, 2,2'-sulfonylbis-, dimethyl ester |
| 36    | 11.44  | 154022 | 1.01  | 61984  | 2.04    | Copaene  |
| 37    | 11.67  | 179442 | 1.18  | 13173  | 0.43    | Campesterol                                    |
| 38    | 11.89  | 45173  | 0.30  | 14509  | 0.48    | Caryophyllene                                  |
| 39    | 12.05  | 44144  | 0.29  | 5481   | 0.18    | Benzamide, 2-hydroxy-N-[2-[(4-nitrophenyl      |
| 40    | 12.22  | 36730  | 0.24  | 9121   | 0.30    | Cyclohexane, 1-ethenyl-1-methyl-2-(1-meth      |
| 41    | 12.51  | 62406  | 0.41  | 17374  | 0.57    | Naphthalene, decahydro-4a-methyl-1-methy       |
| 42    | 12.57  | 74157  | 0.49  | 20468  | 0.67    | Caryophyllene-(I1)                             |
| 43    | 12.65  | 35937  | 0.24  | 11669  | 0.38    | Beta-methoxycinnamic acid                      |
| 44    | 12.74  | 21907  | 0.14  | 9222   | 0.30    | Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethy    |
| 45    | 12.80  | 60315  | 0.39  | 20526  | 0.67    | (-)-.alpha.-Panasinsen                         |
| 46    | 12.88  | 26460  | 0.17  | 10157  | 0.33    | Tetradecanoic acid                             |
| 47    | 12.94  | 110285 | 0.72  | 27975  | 0.92    | 2,5-Dihydro-2-ethyl-2,4-dimethyl-5-oxofura     |
| 48    | 13.03  | 188783 | 1.24  | 33293  | 1.09    | 1,3-Benzenediol, 4-propyl-                     |
| 49    | 13.41  | 18993  | 0.12  | 4720   | 0.16    | 1-Dimethyl(ethenyl)silyloxy-2-propene          |
| 50    | 13.53  | 38746  | 0.25  | 11790  | 0.39    | Methyl-(2-hydroxy-3-ethoxy-benzyl)ether        |

|     |        |         |       |        |       |  |
|-----|--------|---------|-------|--------|-------|--|
| 51  | 13.62  | 145315  | 0.95  | 31307  | 1.03  | 3-Furoic acid, tert-butyl dimethylsilyl ester                  |
| 52  | 13.74  | 96974   | 0.64  | 18380  | 0.60  | 5,9-Tetradecadienedioic acid, 5,6,9,10-tetra                   |
| 53  | 13.84  | 103268  | 0.68  | 24284  | 0.80  | 3-Furoic acid, tert-butyl dimethylsilyl ester                  |
| 54  | 14.01  | 40942   | 0.27  | 13229  | 0.43  | Cyclopentanecarboxylic acid, 2-oxo-, methyl                    |
| 55  | 14.12  | 151466  | 0.99  | 44816  | 1.47  | Methyl 2,4,6-trihydroxybenzoate                                |
| 56  | 14.33  | 46654   | 0.31  | 8969   | 0.29  | 2-Hexenedioic acid, 2-methoxy-, dimethyl e                     |
| 57  | 14.47  | 87287   | 0.57  | 14970  | 0.49  | 1,3-Dimethyl-1,3-di(but-3-enyl)1,3-disilacyc                   |
| 58  | 14.54  | 19496   | 0.13  | 10887  | 0.36  | myristic acid  |
| 59  | 14.62  | 33699   | 0.22  | 8183   | 0.27  | Pyridin-3-ol, O-acetyl-2-[S-[2-acetoxyethyl]d                  |
| 60  | 14.69  | 48217   | 0.32  | 5908   | 0.19  | 2-Hydroxy-5-methylisophthalaldehyde                            |
| 61  | 14.91  | 33336   | 0.22  | 10178  | 0.33  | Thieno[2,3-c]furan-3-carbonitrile, 2-amino-                    |
| 62  | 15.37  | 27271   | 0.18  | 11080  | 0.36  | 1,2-Benzenedicarboxylic acid, bis(2-methylp                    |
| 63  | 15.47  | 21552   | 0.14  | 5408   | 0.18  | 1,3-Dithiolane, 2-methyl-2-phenyl-                             |
| 64  | 16.11  | 52809   | 0.35  | 13706  | 0.45  | Z-11-Pentadecenol  |
| 65  | 16.32  | 979582  | 6.41  | 285349 | 9.37  | l-(+)-Ascorbic acid 2,6-dihexadecanoate                        |
| 66  | 16.69  | 23424   | 0.15  | 9646   | 0.32  | Pentadecanoic acid, ethyl ester                                |
| 67  | 17.33  | 33401   | 0.22  | 6727   | 0.22  | Heptadecanoic acid   |
| 68  | 17.68  | 26787   | 0.18  | 7811   | 0.26  | n-Pentadecanol   |
| 69  | 17.77  | 41954   | 0.27  | 11528  | 0.38  | 9,12-Octadecadienoic acid (Z,Z)-, methyl es                    |
| 70  | 17.87  | 42725   | 0.28  | 11140  | 0.37  | 7-Octadecenoic acid, methyl ester                              |
| 71  | 18.20  | 724227  | 4.74  | 165903 | 5.45  | cis-11,14-Eicosadienoic acid, methyl ester                     |
| 72  | 18.34  | 4325023 | 28.32 | 713350 | 23.43 | Octadec-9-enoic acid   |
| 73  | 18.63  | 1412950 | 9.25  | 313547 | 10.30 | Octadecanoic acid  |
| 74  | 18.75  | 164133  | 1.07  | 35811  | 1.18  | Ethyl Oleate   |
| 75  | 18.94  | 47882   | 0.31  | 10479  | 0.34  | 1H-1,2,3-Triazole-4-carboxylic acid, 5-pent 2,4 dichlorophenon |
| 76  | 19.0   | 34128   | 0.22  | 7437   | 0.24  | 2,4-Dichloro-2',4'-dimethylbenzophenone                        |
| 77  | 19.14  | 40501   | 0.27  | 10382  | 0.34  | Heptadecanoic acid, 15-methyl-, ethyl ester                    |
| 78  | 20.125 | 46869   | 0.31  | 5707   | 0.19  | Pyrrolidine, 1-(1,6-dioxooctadecyl                             |
| 79  | 20.35  | 57432   | 0.38  | 8726   | 0.29  | Squalane   |
| 80  | 22.50  | 20767   | 0.14  | 4317   | 0.14  | Tricyclo[3.3.1.1(3,7)]decan-6-one, 2,2,7-tri                   |
| 81  | 23.15  | 31368   | 0.21  | 4274   | 0.14  | N-(4-Acetylamino-phenyl)-4-chloro-3-nitro-                     |
| 82  | 25.50  | 89259   | 0.58  | 19002  | 0.62  | 1,2-Benzenedicarboxylic acid, diisooctyl est                   |
| 83  | 26.61  | 35341   | 0.23  | 6742   | 0.22  | Methyl 3-bromobenzo(b)thiophene-6-carbox                       |
| 84  | 26.87  | 20046   | 0.13  | 7976   | 0.26  | 2-Ethylamino-6,7-dihydro-8-isopropylimida                      |
| 85  | 27.10  | 117989  | 0.77  | 10495  | 0.34  | Ergosta-7,22-dien-3-ol, (3.beta.,5.alpha.,22E                  |
| 86  | 27.51  | 46039   | 0.30  | 10948  | 0.36  | Dicyclooctanopyridazine  |
| 87  | 27.60  | 53337   | 0.35  | 14150  | 0.46  | 9-Octadecenoic acid (Z)-, 2,3-dihydroxyprop                    |
| 88  | 28.04  | 21265   | 0.14  | 7185   | 0.24  | Octadecanoic acid, 2,3-dihydroxypropyl este                    |
| 89  | 29.32  | 41858   | 0.27  | 4451   | 0.15  | Adenosine, N(6)-[1-phenethyl]-4'-[N-ethylca                    |
| 90  | 29.49  | 90363   | 0.59  | 12680  | 0.42  | Cholesterol  |
| 91  | 31.37  | 43983   | 0.29  | 9466   | 0.31  | 1H,3H-Quinoline-2,5-dione, 1-(4-fluorophe                      |
| 92  | 31.45  | 38338   | 0.25  | 8809   | 0.29  | Stigmasta-4,22-dien-3.beta.-ol                                 |
| 93  | 32.02  | 72110   | 0.47  | 15162  | 0.50  | .beta.-Amyrene   |
| 94  | 32.10  | 117162  | 0.77  | 16176  | 0.53  | :Lup-20(29)-en-3-one \$\$ Lup-20(30)-en-3-one                  |
| 95  | 32.29  | 171883  | 1.13  | 27693  | 0.91  | 2-Propanone, 1-[[4-[(4-bromophenyl)imino]                      |
| 96  | 32.46  | 47659   | 0.31  | 8924   | 0.29  | d-Glucitol, 1-S-decyl-1-thio-                                  |
| 97  | 33.57  | 31077   | 0.20  | 3615   | 0.12  | gamma.-Sitosterol  |
| 98  | 33.67  | 45515   | 0.30  | 11593  | 0.38  | Resorcinol, bis(tert-butyl dimethylsilyl) ether                |
| 99  | 33.86  | 148407  | 0.97  | 27553  | 0.91  | 2-(3,7-Dimethyl-octa-2,6-dienyl)-4-methoxy                     |
| 100 | 34.31  | 296273  | 1.94  | 28054  | 0.92  | Ethanethioic acid, S-heptadecyl ester                          |

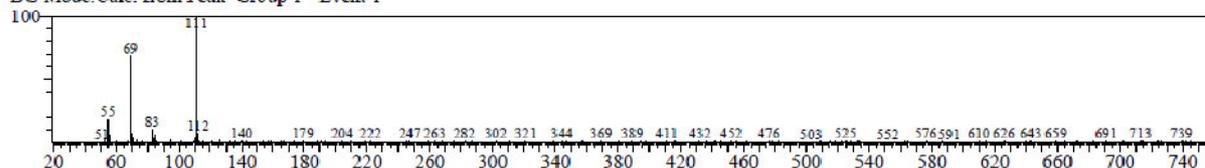
**Table-3: Some of the components identified in the methanolic extracts of *Moringa oleifera* with their molecular formula, molecular weight, nature and biological activity**

| Compound Name   | RT     | Peak Area % | Molecular Formula   | Molecular Weight g/mol | Compound nature  |
|---|--------|-------------|---|------------------------|--|
| 2-Pyrazoline, 1-isopropyl-5-methyl-                               | 3.67   | 0.03        | C <sub>7</sub> H <sub>14</sub> N <sub>2</sub>                 | 126                    | Pyrazoline alkaloid  |
| 4-Ethoxy-6-piperidin-1-yl-[1,3,5]triazine-2-carboxylic acid amide | 4.67   | 0.17        | C <sub>11</sub> H <sub>17</sub> N <sub>5</sub> O <sub>2</sub> | 251                    | Piperidin alkaloid   |
| Neocurdione   | 5.38   | 0.14        | C <sub>15</sub> H <sub>24</sub> O <sub>2</sub>                | 236                    | Cytotoxic Sesquiterpenoid  |
| 2-Exo-hydroxy-5-ketobornane                                       | 6.77   | 0.15        | C <sub>10</sub> H <sub>16</sub> O <sub>2</sub>                | 168                    | Bicyclic terpenoid   |
| Piperazine, 1,4-dimethyl  | 6.84   | 0.45        | C <sub>6</sub> H <sub>14</sub> N <sub>2</sub>                 | 114                    | Preparation product and raw material , have very important role in radical cyclizations  |
| 6,6-Dimethoxy-piperidin-2-one                                     | 7.19   | 0.22        | C <sub>7</sub> H <sub>13</sub> NO <sub>3</sub>                | 159                    | Piperidin alkaloid   |
| Quinoline, 3-methyl   | 10.94  | 1.18        | C <sub>10</sub> H <sub>9</sub> N                              | 143                    | Quinoline alkaloid   |
| Copaene   | 11.44  | 1.01        | C <sub>15</sub> H <sub>24</sub>                               | 204                    | Sesquiterpene essential oil  |
| Campesterol   | 11.67  | 1.18        | C <sub>28</sub> H <sub>48</sub> O                             | 401                    | Steroidal compound   |
| Myristic acid   | 14.54  | 0.13        | C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>                | 228                    | Saturated fatty acid   |
| Coumaric acid   | 14.69  | 0.32        | C <sub>9</sub> H <sub>8</sub> O <sub>3</sub>                  | 164                    | Plant metabolite with antioxidant and anti-inflammatory properties   |
| Linoleic acid   | 17.77  | 0.27        | C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>                | 294                    | Unsaturated omega-6 fatty acid   |
| Oleic acid  | 18.34  | 28.32       | C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>                | 282                    | Monounsaturated omega-9 fatty acid   |
| Stearic acid  | 18.63  | 9.25        | C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>                | 284                    | Saturated fatty acid uses as a surfactant and softening agent  |
| Arachidic acid  | 19.14  | 0.27        | C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>                | 312                    | Saturated fatty acid. surfactant-like properties, it is used in the manufacture of pharmaceuticals, soaps, cosmetics, and food packaging.  |
| Pyrrolidine, 1-(1,6-dioxooctadecyl)-                              | 20.125 | 0.31        | C <sub>22</sub> H <sub>41</sub> NO <sub>2</sub>               | 351                    | Alkaloid   |
| Squalane  | 20.358 | 0.38        | C <sub>30</sub> H <sub>62</sub>                               | 422                    | Hydrocarbon and tri-terpene derived by hydrogenation of squalene It's also an antioxidant-rich, age-fighting emollient that's commonly used as an additive in deodorants, lip balm, lipstick, moisturizers, sun tan lotions, supplements and a variety of other cosmetic |
| Alpha-ergosterol  | 27.10  | 0.77        | C <sub>28</sub> H <sub>46</sub> O                             | 398                    | Biological precursor provitamin to vitamin D <sub>2</sub>  |
| Cholesterol   | 29.49  | 0.59        | C <sub>27</sub> H <sub>46</sub> O                             | 386                    | Sterol, serves as a precursor for the biosynthesis of steroid hormones, bile acids, and vitamin D  |
| Stigmasta-4,22-dien-3.beta.-ol                                    | 31.45  | 0.25        | C <sub>29</sub> H <sub>48</sub> O                             | 412                    | Sterol, used as a precursor in the manufacture of semisynthetic progesterone   |
| Beta-Amyrene  | 32.02  | 0.47        | C <sub>30</sub> H <sub>50</sub>                               | 410                    | Triterpene compound  |
| Lupenon   | 32.10  | 0.77        | C <sub>30</sub> H <sub>48</sub> O                             | 424                    | Triterpene compound  |
| Gama-sitosterol   | 33.57  | 0.2         | C <sub>29</sub> H <sub>50</sub> O                             | 414                    | Plant Sterol, serves as a precursor for the biosynthesis of steroid hormones, bile acids, and vitamin D  |

The following charts represent mass fragmentation of some active components identified in the methanolic extract of *Moringa oleifera* and listed in table-3 respectively:

<< Target >>

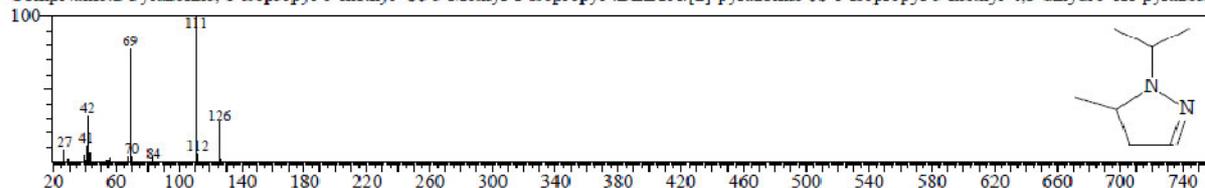
Line# 6 R.Time:3.667(Scan#:81) MassPeaks:333  
RawMode:Averaged 3 658-3 675(80-82) BasePeak:111 10(4600)  
BG Mode:Calc. from Peak Group 1 - Event 1



Hit#:1 Entry:6471 Library:NIST08.LIB

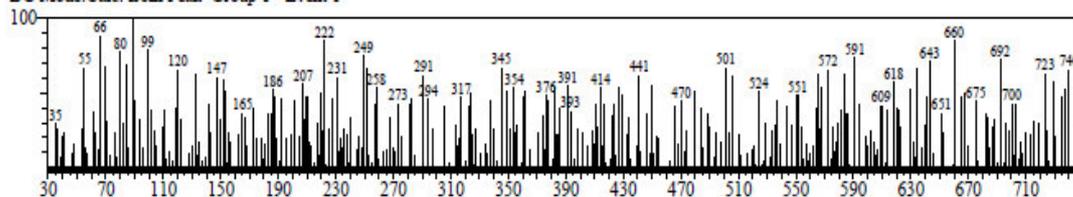
SI:82 Formula:C7H14N2 CAS:26964-54-5 MolWeight:126 RefIndex:929

CompName:2 Pyrazoline, 1 isopropyl 5 methyl \$S\$ 5 Methyl 1 isopropyl .DELTA.[2] pyrazoline \$S\$ 1 Isopropyl 5 methyl 1,5 dihydro 1H pyrazole



<< Target >>

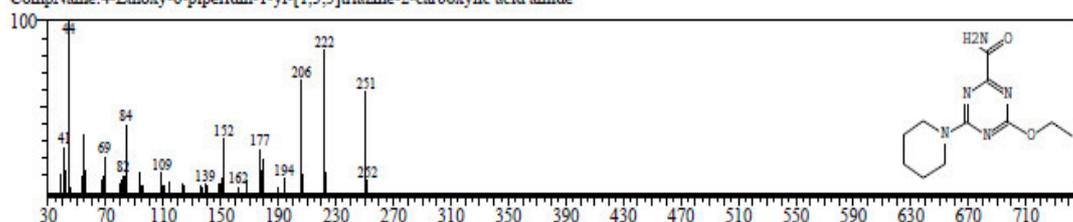
Line# 7 R.Time:4.667(Scan#:201) MassPeaks:336  
RawMode:Averaged 4 658-4 675(200-202) BasePeak:89.00(75)  
BG Mode:Calc. from Peak Group 1 - Event 1



Hit#:1 Entry:76671 Library:NIST08.LIB

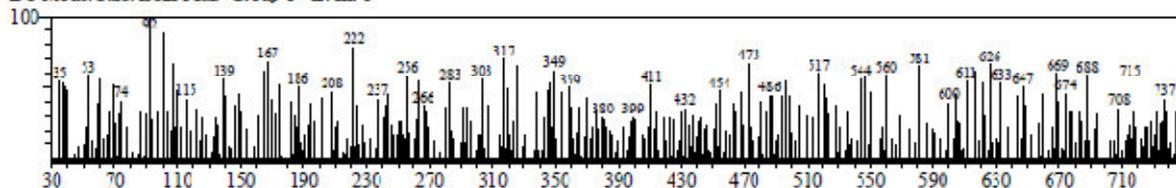
SI:19 Formula:C11H17N5O2 CAS:0-00-0 MolWeight:251 RefIndex:2199

CompName:4-Ethoxy-6-piperidin-1-yl-[1,3,5]triazine-2-carboxylic acid amide

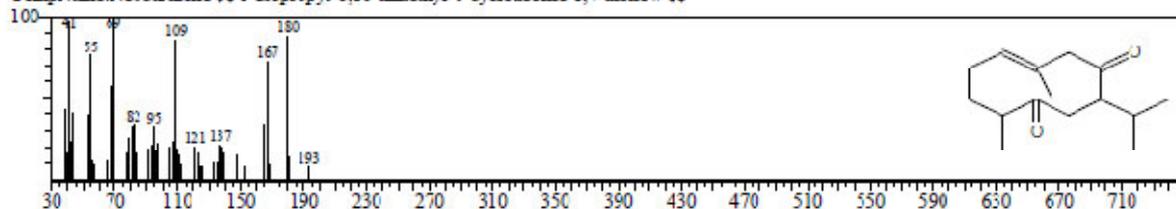


<< Target >>

Line# 9 R.Time:5.385(Scan#:287) MassPeaks:407  
 RawMode:Averaged 5.375-5.392(286-288) BasePeak:92.10(97)  
 BG Mode:Calc. from Peak Group 1 - Event 1

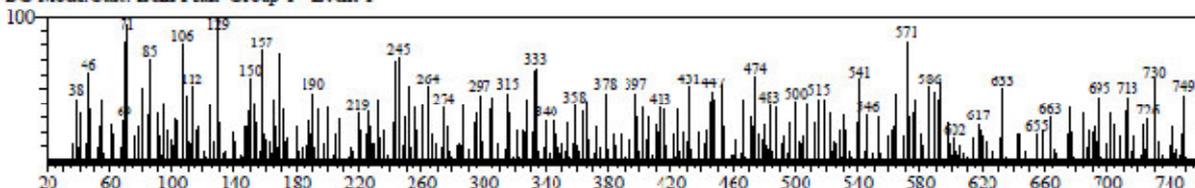


Hit#:1 Entry:66703 Library:NIST08.LIB  
 SI:21 Formula:C15H24O2 CAS:108944-67-8 MolWeight:236 RetIndex:1870  
 CompName:Neocurdione \$\$ 3-Isopropyl-6,10-dimethyl-6-cyclodecene-1,4-dione # \$\$

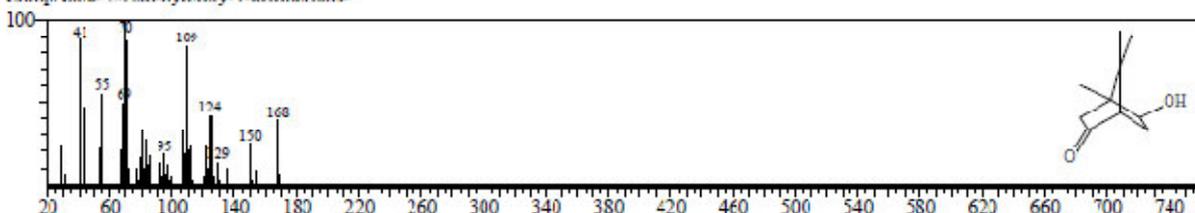


<< Target >>

Line# 17 R.Time:6.767(Scan#:453) MassPeaks:359  
 RawMode:Averaged 6.758-6.775(452-454) BasePeak:129.10(84)  
 BG Mode:Calc. from Peak Group 1 - Event 1

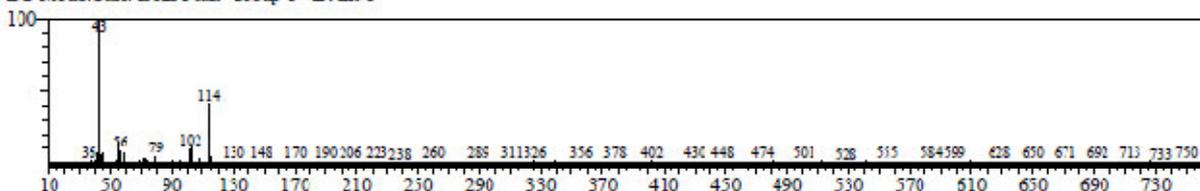


Hit#:1 Entry:24225 Library:NIST08.LIB  
 SI:21 Formula:C10H16O2 CAS:0-00-0 MolWeight:168 RetIndex:1309  
 CompName:2-Exo-hydroxy-5-kestohemara

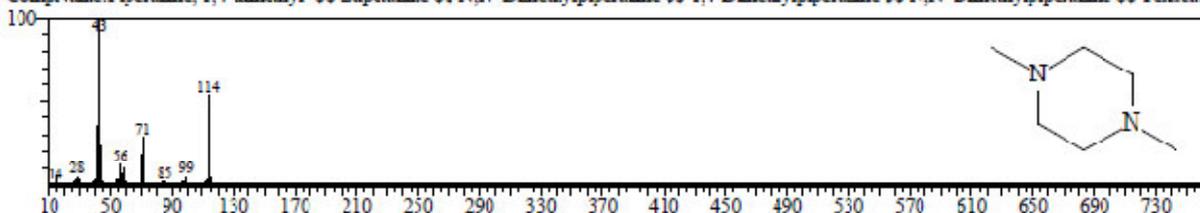


<< Target >>

Line# 18 R.Time:6.833(Scan#:461) MassPeaks:384  
 RawMode:Averaged 6.825-6.842(460-462) BasePeak:43.10(4153)  
 BG Mode:Calc. from Peak Group 1 - Event 1

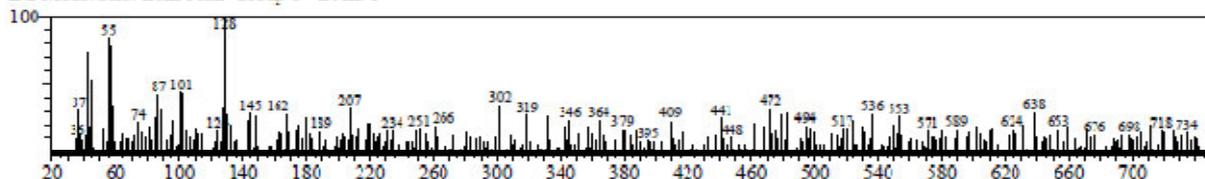


Hit#:1 Entry:4069 Library:NIST08.LIB  
 SI:72 Formula:C6H14N2 CAS:106-58-1 MolWeight:114 RetIndex:946  
 CompName:Piperazine, 1,4-dimethyl- \$\$ Lupetazine \$\$ N,N-Dimethylpiperazine \$\$ 1,4-Dimethylpiperazine \$\$ N,N-Dimethylpiperazine \$\$ Texcat

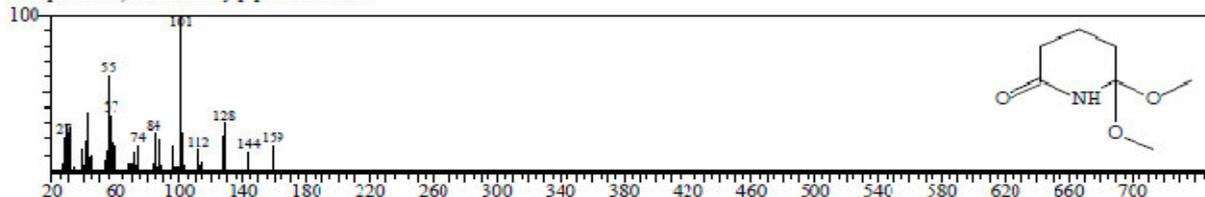


<< Target >>

Line#:20 R.Time:7.192(Scan#:504) MassPeaks:384  
 RawMode:Averaged 7.183-7.200(503-505) BasePeak:128.20(225)  
 BG Mode:Calc. from Peak Group 1 - Event 1

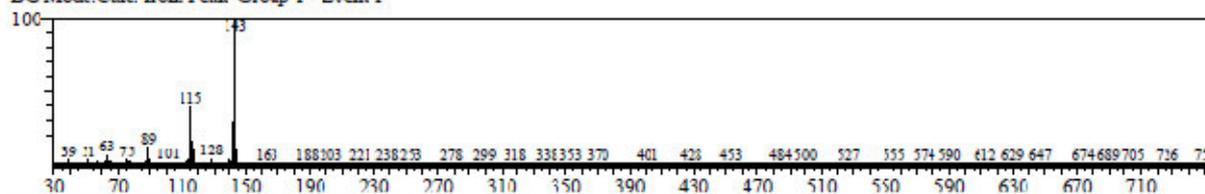


Hit#:1 Entry:19531 Library:NIST08.LIB  
 SI:43 Formula:C7H13NO3 CAS:0-00-0 MolWeight:159 RetIndex:1169  
 CompName:6,6-Dimethoxy-piperidin-2-one

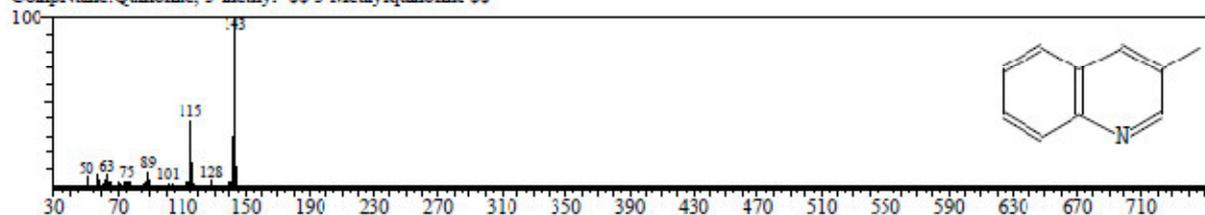


<< Target >>

Line#:33 R.Time:10.942(Scan#:954) MassPeaks:358  
 RawMode:Averaged 10.933-10.950(953-955) BasePeak:143.20(18901)  
 BG Mode:Calc. from Peak Group 1 - Event 1

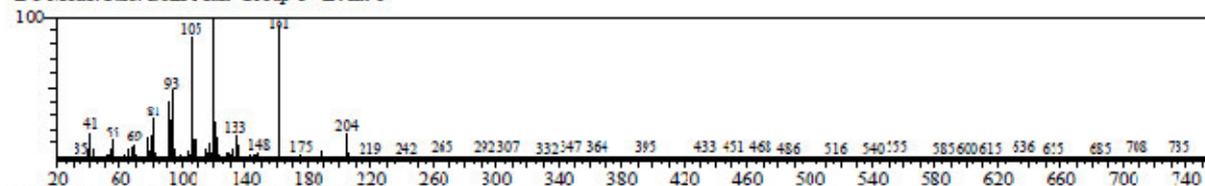


Hit#:1 Entry:12528 Library:NIST08.LIB  
 SI:96 Formula:C10H9N CAS:612-58-8 MolWeight:143 RetIndex:1338  
 CompName:Quinoline, 3-methyl- \$\$\$\$ 3-Methylquinoline \$\$\$\$

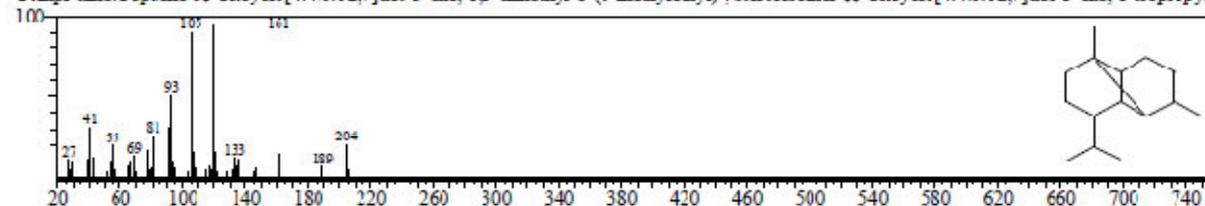


<< Target >>

Line#:36 R.Time:11.442(Scan#:1014) MassPeaks:399  
 RawMode:Averaged 11.433-11.450(1013-1015) BasePeak:119.20(6904)  
 BG Mode:Calc. from Peak Group 1 - Event 1

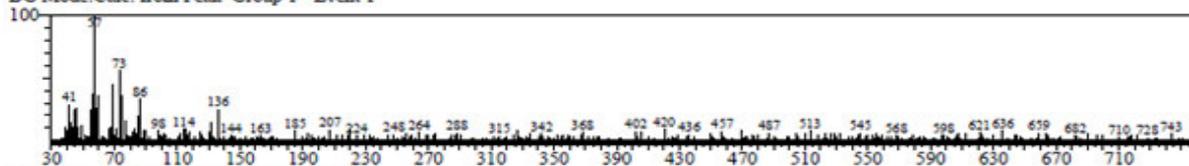


Hit#:1 Entry:15521 Library:NIST08.LIB  
 SI:94 Formula:C15H24 CAS:3856-25-5 MolWeight:204 RetIndex:1221  
 CompName:Coprene \$\$\$\$ Tricyclo[4.4.0.0(2,7)]dec-3-ene, 1,3-dimethyl-8-(1-methylethyl)-, stereoisomer \$\$\$\$ Tricyclo[4.4.0.0(2,7)]dec-3-ene, 8-isopropyl-

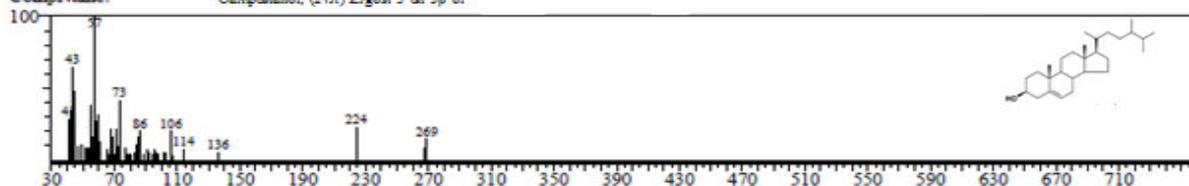


<< Target >>

Line#:37 R.Time:11.675(Scan#:1042) MassPeaks:369  
 RawMode:Averaged 11.667-11.683(1041-1043) BasePeak:57.10(808)  
 BG Mode:Calc. from Peak Group 1 - Event 1

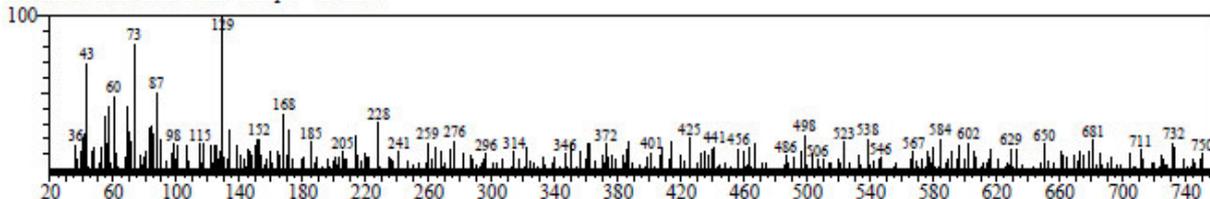


Hit#:1 Entry:168168 Library:NIST08.LIB  
 SI:67 Formula:C<sub>28</sub>H<sub>48</sub>O CAS:96760-57-5 MolWeight:401 RetIndex:3731  
 CompName: Campanolol (21α) Ergost 5 on 3β ol

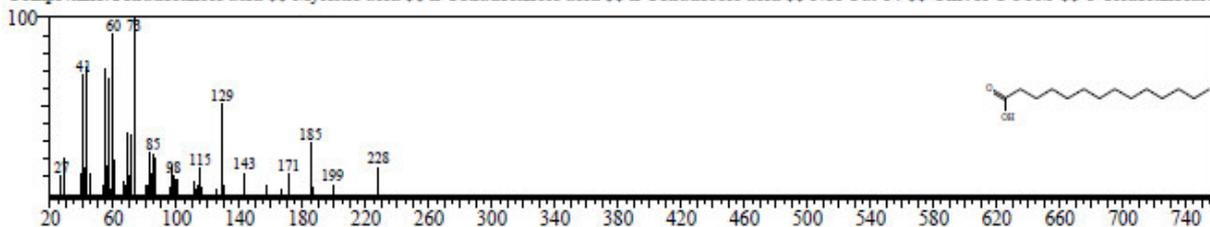


<< Target >>

Line#:58 R.Time:14.542(Scan#:1386) MassPeaks:327  
 RawMode:Averaged 14.533-14.550(1385-1387) BasePeak:129.25(196)  
 BG Mode:Calc. from Peak Group 1 - Event 1

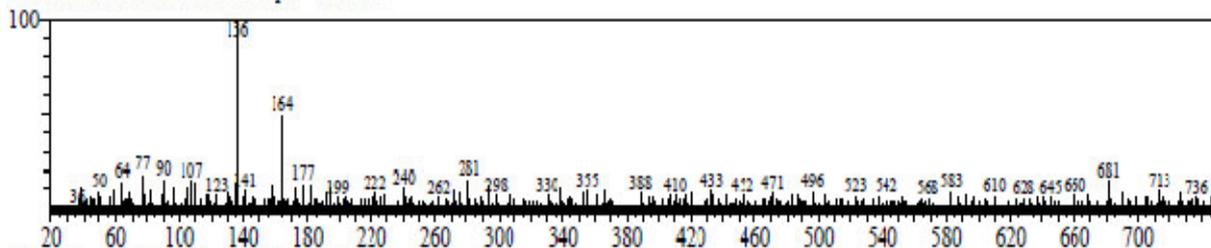


Hit#:1 Entry:61604 Library:NIST08.LIB  
 SI:62 Formula:C<sub>14</sub>H<sub>28</sub>O<sub>2</sub> CAS:544-63-8 MolWeight:228 RetIndex:1769  
 CompName:Tetradecanoic acid \$\$ Myristic acid \$\$ n-Tetradecanoic acid \$\$ n-Tetradecoic acid \$\$ Neo-Fat 14 \$\$ Univol U 316S \$\$ 1-Tridecanecarb



<< Target >>

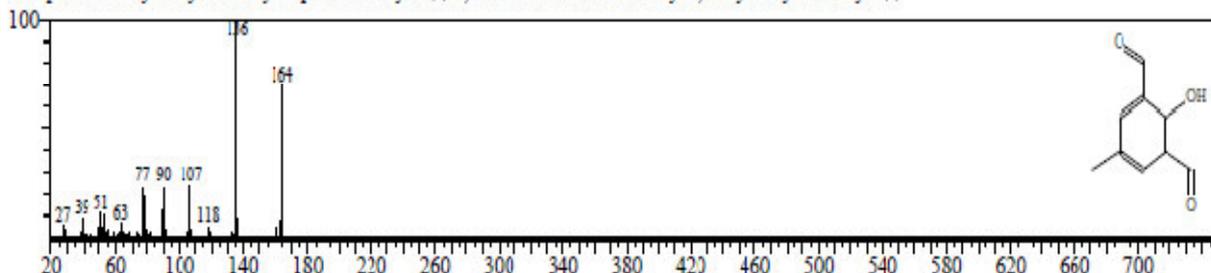
Line#:60 R.Time:14.692(Scan#:1404) MassPeaks:400  
RawMode:Averaged 14.683-14.700(1403-1405) BasePeak:136.20(626)  
BG Mode:Calc. from Peak Group 1 - Event 1



Hit#:1 Entry:21842 Library:NIST08.LIB

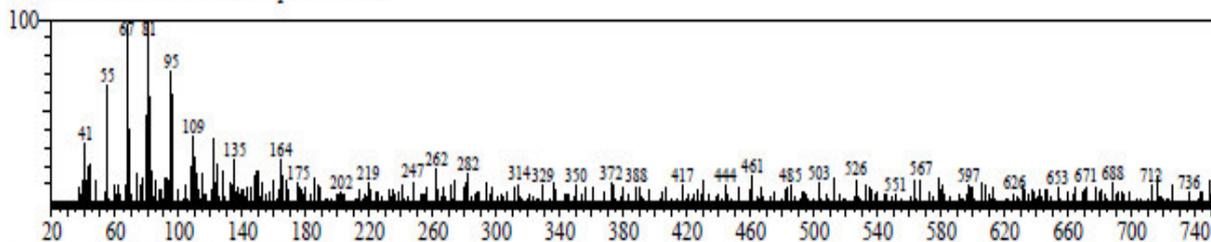
SI:53 Formula:C9H8O3 CAS:7310-95-4 MolWeight:164 RetIndex:1617

CompName:2-Hydroxy-5-methylisophthalaldehyde \$\$ 1,3-Benzenedicarboxaldehyde, 2-hydroxy-5-methyl- \$\$



<< Target >>

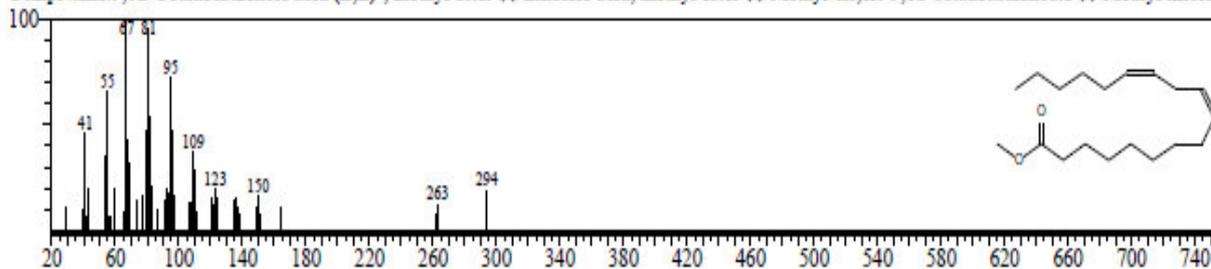
Line#:69 R.Time:17.775(Scan#:1774) MassPeaks:384  
RawMode:Averaged 17.767-17.783(1773-1775) BasePeak:81.15(432)  
BG Mode:Calc. from Peak Group 1 - Event 1



Hit#:1 Entry:107904 Library:NIST08.LIB

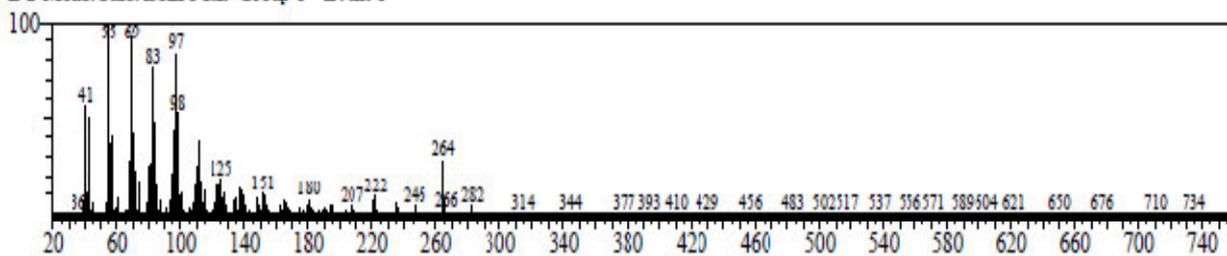
SI:77 Formula:C19H34O2 CAS:112-63-0 MolWeight:294 RetIndex:2093

CompName:9,12-Octadecadienoic acid (Z,Z)-, methyl ester \$\$ Linoleic acid, methyl ester \$\$ Methyl cis,cis-9,12-octadecadienoate \$\$ Methyl linoleat

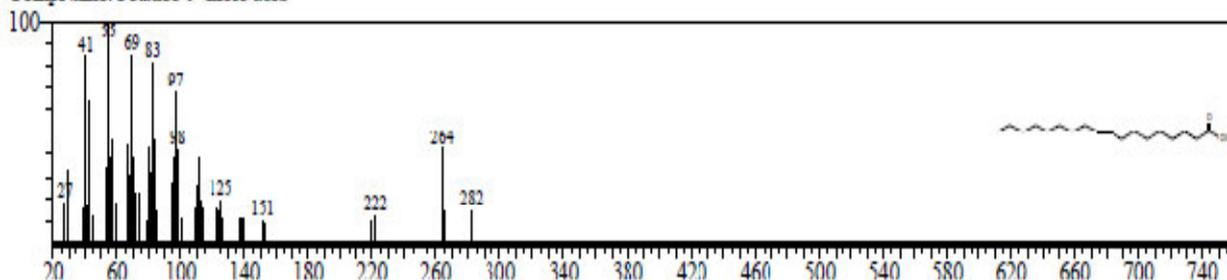


<< Target >>

Line#:72 R.Time:18.333(Scan#:1841) MassPeak:446  
RawMode:Averaged 18.325-18.342(1840-1842) BasePeak:55.10(38120)  
BG Mode:Calc. from Peak Group 1 - Event 1

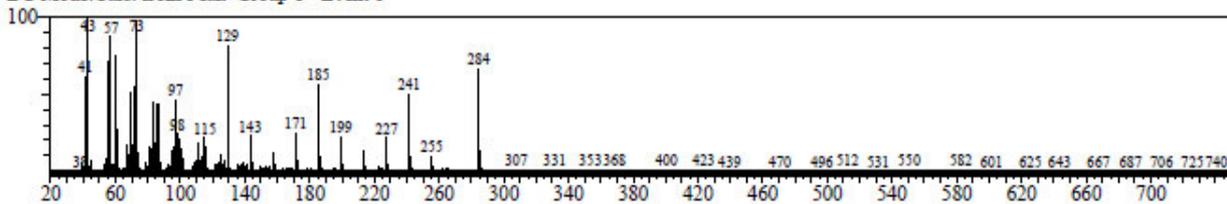


Hit#:1 Entry:99163 Library:NIST08.LIB  
SI:91 Formula:C18H34O2 CAS:0-00-0 MolWeight:282 RetIndex:2175  
CompName:Octadec-9-enoic acid

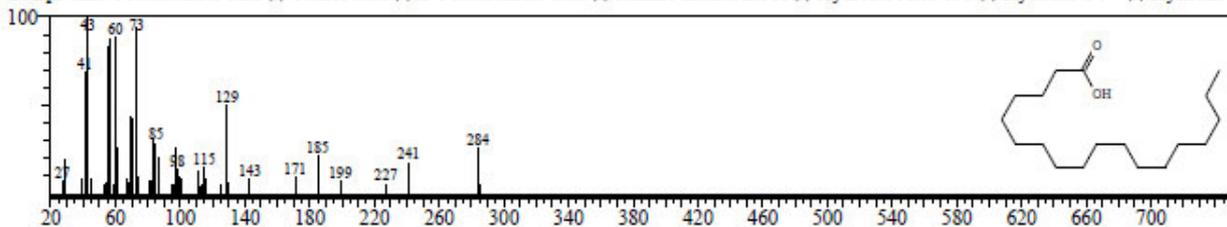


<< Target >>

Line#:73 R.Time:18.625(Scan#:1876) MassPeak:469  
RawMode:Averaged 18.617-18.633(1875-1877) BasePeak:43.10(15222)  
BG Mode:Calc. from Peak Group 1 - Event 1

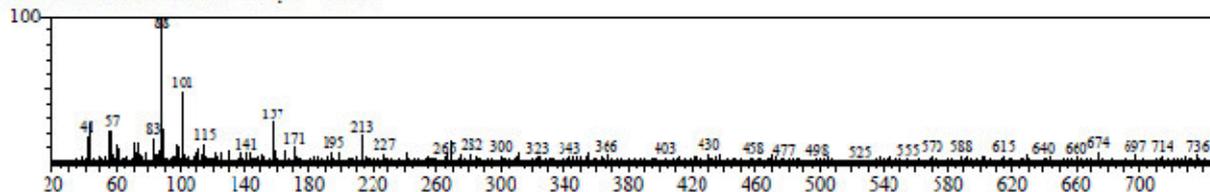


Hit#:1 Entry:100791 Library:NIST08.LIB  
SI:88 Formula:C18H36O2 CAS:57-11-4 MolWeight:284 RetIndex:2167  
CompName:Octadecanoic acid \$\$ Stearic acid \$\$ n-Octadecanoic acid \$\$ Humko Industriene R \$\$ Hydrofol Acid 150 \$\$ Hystrene S-97 \$\$ Hystrene ?

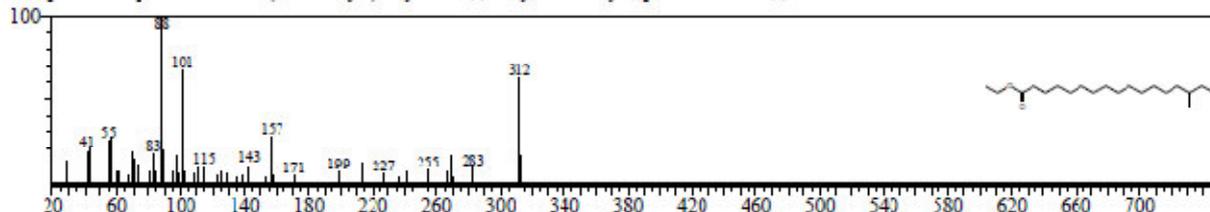


<< Target >>

Line#:77 R.Time:19.142(Scan#:1938) MassPeaks:393  
RawMode:Averaged 19.133-19.150(1937-1939) BasePeak:88.10(947)  
BG Mode:Calc. from Peak Group 1 - Event 1

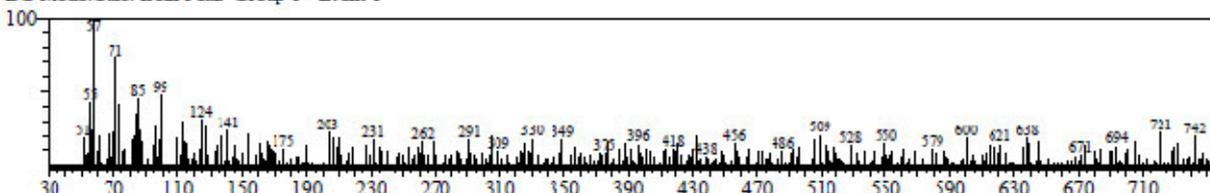


Hit#:1 Entry:120701 Library:NIST08.LIB  
SI:75 Formula:C20H40O2 CAS:57274-46-1 MolWeight:312 RefIndex:2112  
CompName:Heptadecanoic acid, 15-methyl-, ethyl ester \$\$ Ethyl 15-methylheptadecanoate # \$\$

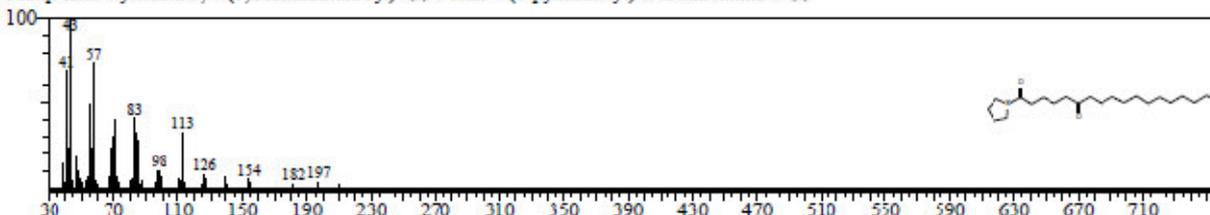


<< Target >>

Line# 113 R.Time:20.125(Scan#:2056) MassPeaks:397  
RawMode:Averaged 20.117-20.133(2055-2057) BasePeak:57.10(482)  
BG Mode:Calc. from Peak Group 1 - Event 1

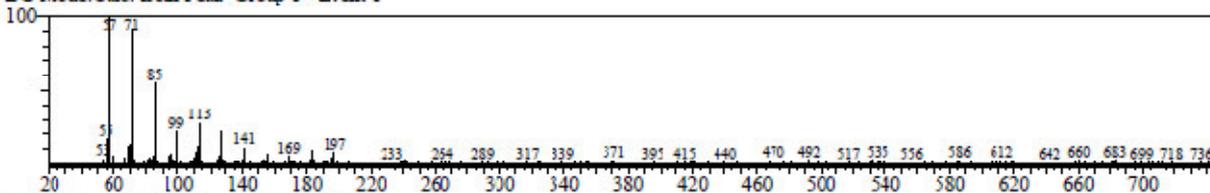


Hit#:1 Entry:145594 Library:NIST08.LIB  
SI:56 Formula:C22H41NO2 CAS:56630-89-8 MolWeight:351 RefIndex:2674  
CompName:Pyrridoline, 1-(1,6-dioxooctadecyl)- \$\$ 1-Octo-(1-(pyrridiny))l-6-octadecanone # \$\$

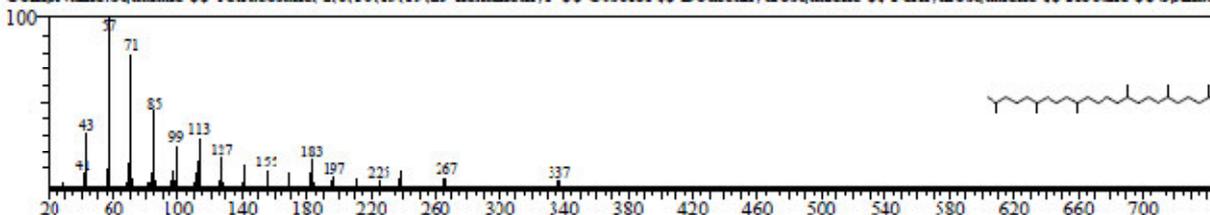


<< Target >>

Line# 115 R.Time:20.358(Scan#:2084) MassPeaks:357  
RawMode:Averaged 20.350-20.367(2083-2085) BasePeak:57.05(3658)  
BG Mode:Calc. from Peak Group 1 - Event 1

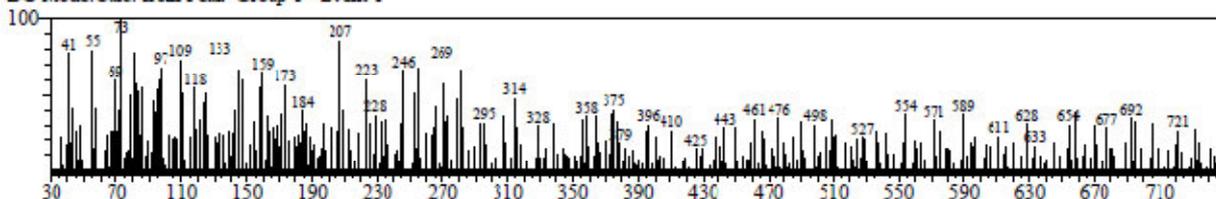


Hit#:1 Entry:173671 Library:NIST08.LIB  
SI:88 Formula:C30H62 CAS:111-01-3 MolWeight:422 RefIndex:2619  
CompName:Squalane \$\$ Tetracosane, 2,6,10,15,19,23-hexamethyl- \$\$ Cosbiol \$\$ Dodecahydrosqualene \$\$ Perhydrosqualene \$\$ Robane \$\$ Spinaca

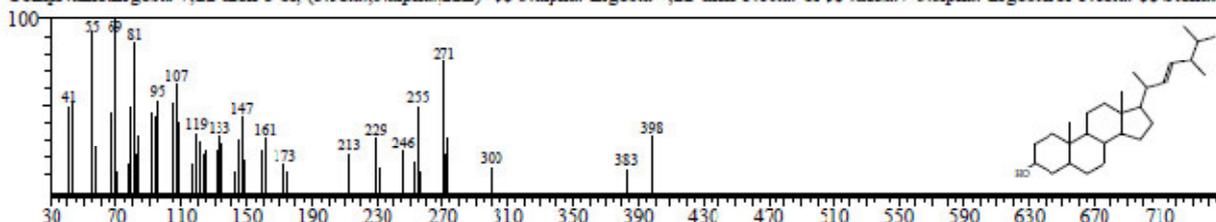


<< Target >>

Line#85 R.Time:27.100(Scan#2893) MassPeaks:392  
 RawMode:Averaged 27.092-27.108(2892-2894) BasePeak:73.10(145)  
 BG Mode:Calc. from Peak Group 1 - Event 1

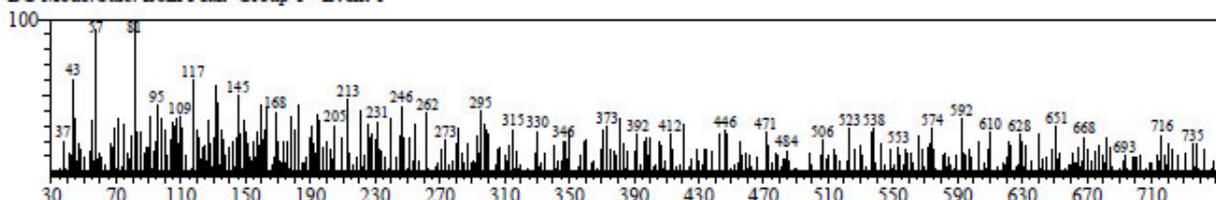


Hit#1 Entry:167348 Library:NIST08.LIB  
 SI:49 Formula:C28H46O CAS:2465-11-4 MolWeight:398 RetIndex:2640  
 CompName:Ergosta-7,22-dien-3-ol, (3.beta.,5.alpha.,22E)- \$\$ 5.alpha.-Ergosta-7,22-dien-3.beta.-ol \$\$ .delta.7-5.alpha.-Ergosterol-3.beta. \$\$ Stellaste

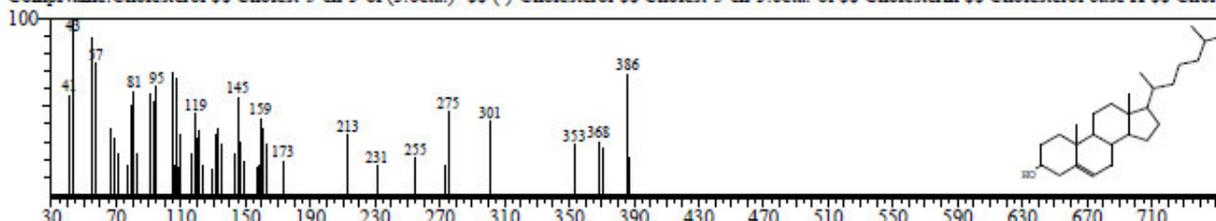


<< Target >>

Line#90 R.Time:29.483(Scan#3179) MassPeaks:415  
 RawMode:Averaged 29.475-29.492(3178-3180) BasePeak:81.15(179)  
 BG Mode:Calc. from Peak Group 1 - Event 1

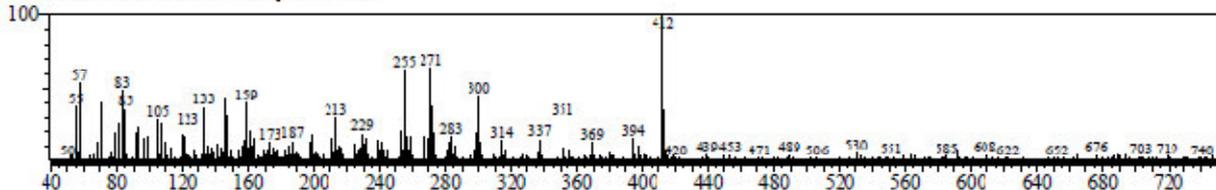


Hit#1 Entry:162891 Library:NIST08.LIB  
 SI:47 Formula:C27H46O CAS:57-88-5 MolWeight:386 RetIndex:2596  
 CompName:Cholesterol \$\$ Cholest-5-en-3-ol (3.beta.)- \$\$ (-)-Cholesterol \$\$ Cholest-5-en-3.beta.-ol \$\$ Cholesterin \$\$ Cholesterol base H \$\$ Cholest

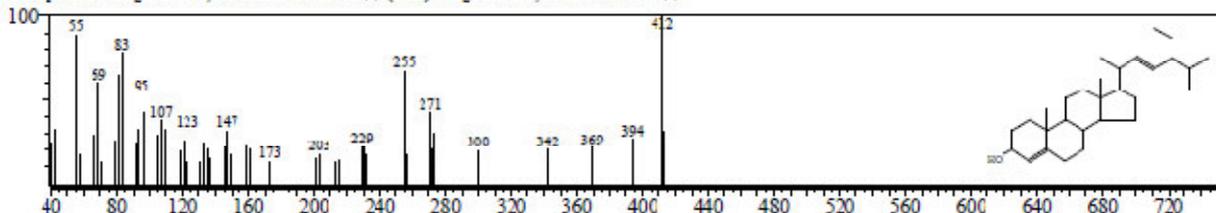


<< Target >>

Line#172 R.Time:29.600(Scan#3193) MassPeaks:406  
 RawMode:Averaged 29.592-29.608(3192-3194) BasePeak:412.30(2095)  
 BG Mode:Calc. from Peak Group 1 - Event 1

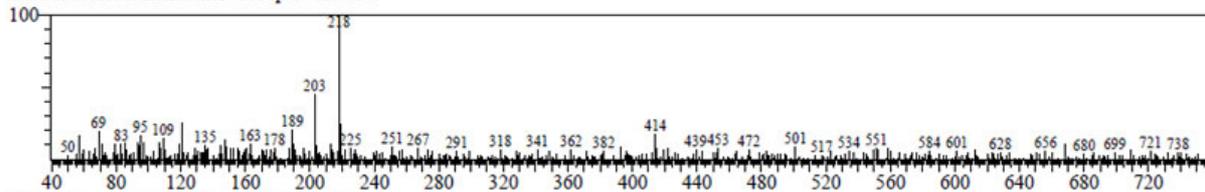


Hit#1 Entry:171296 Library:NIST08.LIB  
 SI:70 Formula:C29H48O CAS:57815-94-8 MolWeight:412 RetIndex:2739  
 CompName:Stigmasta-4,22-dien-3.beta.-ol (22E)-Stigmasta-4,22-dien-3-ol # \$\$

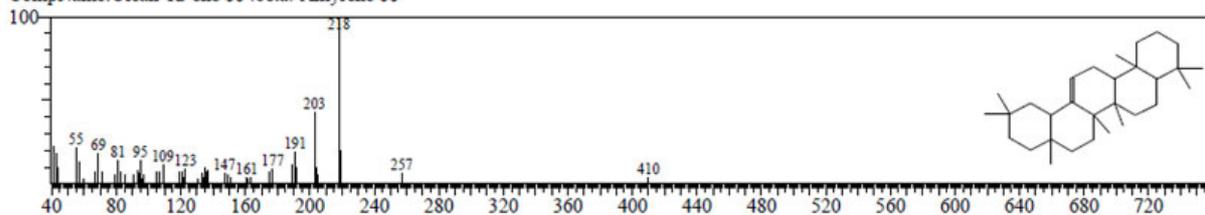


<< Target >>

Line#:188 R.Time: 32.025(Scan#:3447) MassPeaks:404  
RawMode:Averaged 31.708-31.725(3446-3448) BasePeak:218.10(995)  
BG Mode:Calc. from Peak Group 1 - Event 1

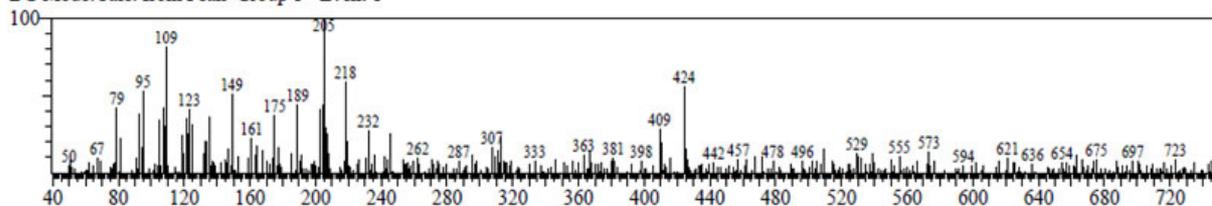


Hit#:1 Entry:170760 Library:NIST08.LIB  
SI:66 Formula:C30H50 CAS:471-68-1 MolWeight:410 RetIndex:2697  
CompName:Olean-12-ene \$\$ .beta.-Amyrene \$\$

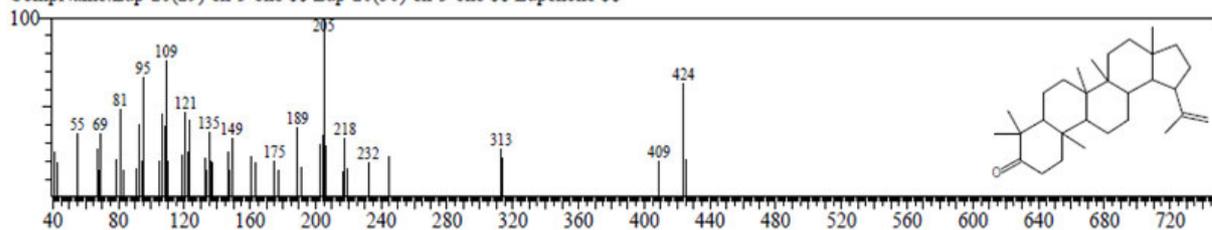


<< Target >>

Line#:191 R.Time: 32.17(Scan#:3507) MassPeaks:377  
RawMode:Averaged 32.208-32.225(3506-3508) BasePeak:205.10(547)  
BG Mode:Calc. from Peak Group 1 - Event 1

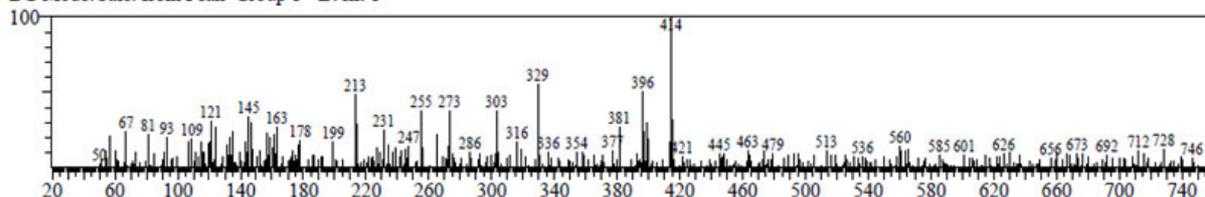


Hit#:1 Entry:174155 Library:NIST08.LIB  
SI:80 Formula:C30H48O CAS:1617-70-5 MolWeight:424 RetIndex:2831  
CompName:Lup-20(29)-en-3-one \$\$ Lup-20(30)-en-3-one \$\$ Lupenone \$\$

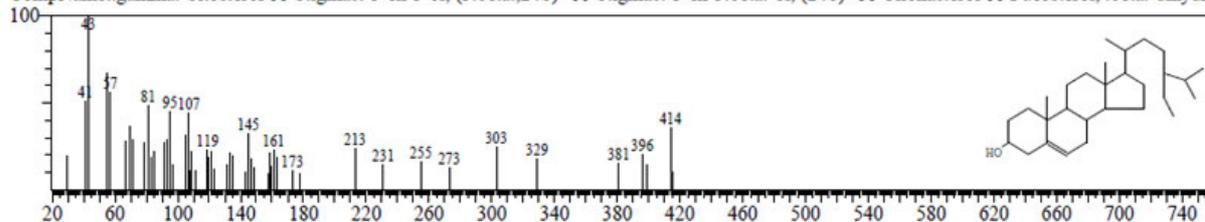


<< Target >>

Line#:187 R.Time:33.561 (Scan#:3426) MassPeaks:396  
RawMode:Averaged 31.533-31.550(3425-3427) BasePeak:414.30(565)  
BG Mode:Calc. from Peak Group 1 - Event 1



Hit#:1 Entry:171855 Library:NIST08.LIB  
SI:61 Formula:C29H50O CAS:83-47-6 MolWeight:414 RetIndex:2731  
CompName: gamma.-Sitosterol \$\$ Stigmast-5-en-3-ol, (3.beta.,24S)- \$\$ Stigmast-5-en-3.beta.-ol, (24S)- \$\$ Clionasterol \$\$ Fucosterol, .beta.-dihydro



## Discussion

Plants have formed the basis for traditional medicinal systems for thousands of years, with the first records dating from about 2600 BC in Mesopotamia. Traditional knowledge of medicinal plants has always guided the search for new cures. In spite of the advent of modern high throughput drug discovery and screening techniques, traditional knowledge systems have given clues to the discovery of valuable drugs.

In the present study, methanolic extract of the *Moringa oleifera* plant cultivated in Iraq was analyzed for the first time. The comparison of the mass spectrum with the NIST database library gave more than 90% match as well as a confirmatory compound structure match. This work will help to identify the compounds, which may be used in body products, drugs, pharmaceutical and therapeutic value since many components isolated from this plant reported for the first time, also the present study results were confirmed the traditional uses of this plant as an antioxidant, anti-inflammatory, antispasmodic, diuretic, antiulcer, flavor agent, antimicrobial, antifungal, pesticide. Based on the results obtained in this study, it could be said that *M. oleifera* plant powder contains chemical constituents of pharmacological and nutritional significance. However, it is recommended that further work be carried out to isolate and purify the bioactive constituents in *M. oleifera* powder using various extraction solvents with a view to characterizing their molecular structure, formula, weight as well as evaluating their safety or otherwise (toxicity) for human and other animal use.

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