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Brown pigment production by *Penicillium. Purpurogenum* using *Moringa oleifera* leaves and identification of phytochemicals by analytical techniques

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Abstract
Water soluble dyes or pigments are of fungal origin, are imparting pleasing and attractive colors to food and textile. These pigments are generally produced from *Moringa. Oleifera.* In the current investigation, the *Penicillium. Purpurogenum* was used for the fermentative production of pigments from fresh leaves of *Moringa. Oleifera.* The optimal conditions for microbial production were at p^H 5.5, temperature 37ºC and production time 7 days. After the production, the pigments were analysed for biologically active phytochemicals by performing spectroscopic techniques like UV-Vis, FTIR, Proton-NMR spectroscopy and Gas Chromatography-Mass Spectrometry.

Keywords: Triclosan, TCS, determination, detection, sensor

1. Introduction
The microbial pigments are remarkable for humans as they are natural sources of colours used in food, textiles, cosmetics, and pharmaceutical industries. Microbial production of pigments is inherently faster and more productive compared to any other chemical process [1]. It is also of high interest owing to its independency of environmental conditions. Microorganisms like bacteria, algae, and fungi produce a variety of pigments and therefore, are the promising source of food colorants [2, 3]. Fungal pigments are secondary metabolites which are not useful in the growth and development of the microorganism but possess vital ecological functions. They have been produced from the secondary metabolism and used as ant-fungal agents. The pigments from microbial sources have also prudent properties like stability to light, heat, and pH [4]. *Moringa. oleifera* Lam. (Moringaceae), also called 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 [5]. *Moringa*, 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 [6]. The edibility of all the parts of the tree and its rich nutritional content contributed to its consumption by humans for many years. The diverse range of medicinal uses for Moringa oleifera, include its use as an antioxidant[7], anti-carcinogenic [8], anti-inflammatory, antispasmodic, diuretic [9], antiulcer, antibacterial, antifungal and its antinociceptive [10] properties, as well as its wound healing ability has been demonstrated [11]. 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 [12].

Phytochemical screening is a process for identifying new bioactive compounds having medicinal significance. The present study aims at the optimal red pigment production by *P. purpurogenum* in a synthetic liquid medium. The spectroscopic analysis of the red pigment was also conducted to establish the potential of the pigment in industrial applications.

2. Materials
- The *Penicillium purpurogenum* - was purchased from NCI Pune, and the stock culture was maintained on a Potato Dextrose Agar (PDA) slants.
- The leaves of *Moringa oleifera* were collected in fresh polythene bags from Kakinada, East Godavari district, A.P.
- PDB (2%), $MgSO_4$ (1%), $MnSO_4$(1%), K_2HPO_4 (1%) and KH_2PO_4 (1%) and Urea(0.5%) with pH 5.5

3. Methodology

3.1 Production of microbial pigments from *Moringa oleifera*

The leaves were initially washed with tap water, followed by distilled water to remove soil and other contaminants. They were weighed and ground into a paste and used as the carbon source.

3.1.1 Fermentation

A loopful of well sporulated culture of the *Penicillium. purpurogenum* was inoculated into a corresponding 250 mL Erlenmeyer flask containing 100 mL of production medium composed of PDB (2%), $MgSO_4$ (1%), $MnSO_4$(1%), K_2HPO_4 (1%) and KH_2PO_4 (1%) and Urea(0.5%) with pH 5.5. The inoculated flask was incubated on a rotary shaker (200 rpm) at 25ºC for 7-10 days. 3.1.2

3.1.2 Pigment extraction

After incubation, the broth obtained was taken and heated on a heating mantle at 70 degrees Celsius for 2 hours. After heating, the broth was filtered, separating the biomass and the filtrate. The pH of the filtrate was checked. The solution obtained was evaporated and concentrated at 70 degrees Celsius. The water molecules are slowly removed on evaporation leaving the solid concentrate. The concentrate was cooled immediately. The crude extract obtained was subjected to crystallization to form crystals of the pigment. The pigment obtained was purified and weighed.

3.2 UV-Visible Spectroscopy (UV-Vis)

The maximum absorbance of extracted and dried red pigment powder was determined by spectrophotometer (SPECORD 210-222K333 UV-Vis) at 500 nm wavelength [12].

3.3. Fourier Transform Infra-Red (FT-IR) spectroscopy

The Fourier transform infrared (FT-IR) spectrum was recorded on a Bruker FT-IR spectrophotometer and the spectral range was 4000 – 500 cm-1**[14].** The dried powder of the red pigment was scanned by a Shimadzu FT-IR 8000 spectrophotometer in the 4000–400 cm−1 range using the K Br method at 27 °C.

3.4. Nuclear Magnetic Resonance Spectroscopy

The purified pigment was dissolved with dimethylsulfoxid e (DMSO d6) and the sample was injected into a nuclear magnetic resonance (NMR) spectrometer (Bruker 400 MHz) [15].

3.5. Gas Chromatography-Mass Spectrometry

In the current study the microbial extract of *M. oleifera* was screened for the presence of phytochemical compounds by qualitative test procedures followed by GC-MS analysis for detection of novel compounds. The mass spectra of compounds in the microbial extract was matched with that of NIST (National Institute Of standards and Technology) and Wiley library.

4. Results and Discussion

4.1. The conditions under which the pigment was produced were p^H 5.5, temperature 37ºC and production time of 8 days. The highest production of *Moringa. oleifera* pigments was 2.8% i.e., 28.5g/L yield and maximum UV absorbance at 224. 39nm (1.4985). The produced pigment was characterized using different techniques like UV Visible Spectroscopy, FTIR and 1H NMR and GC-MS techniques.

UV Spectra of Moringa

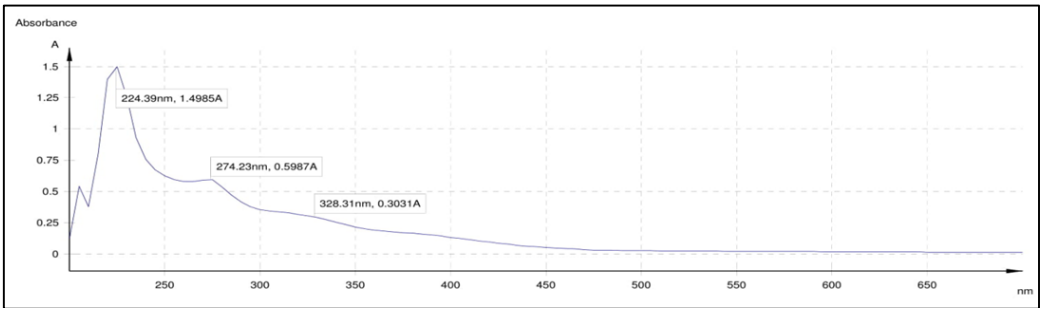
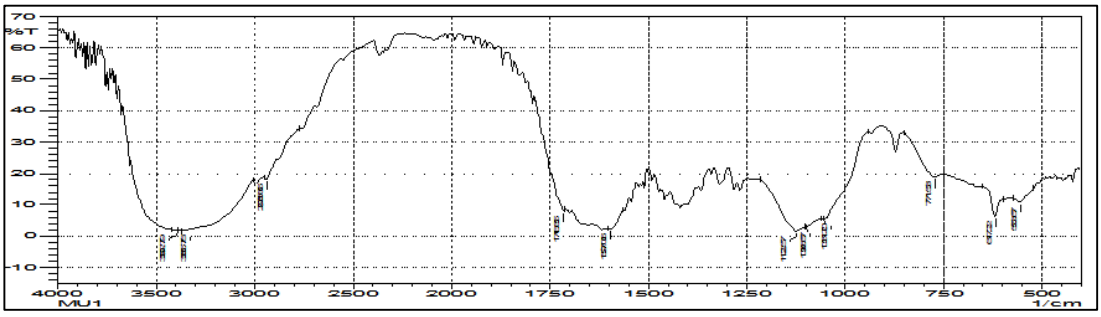


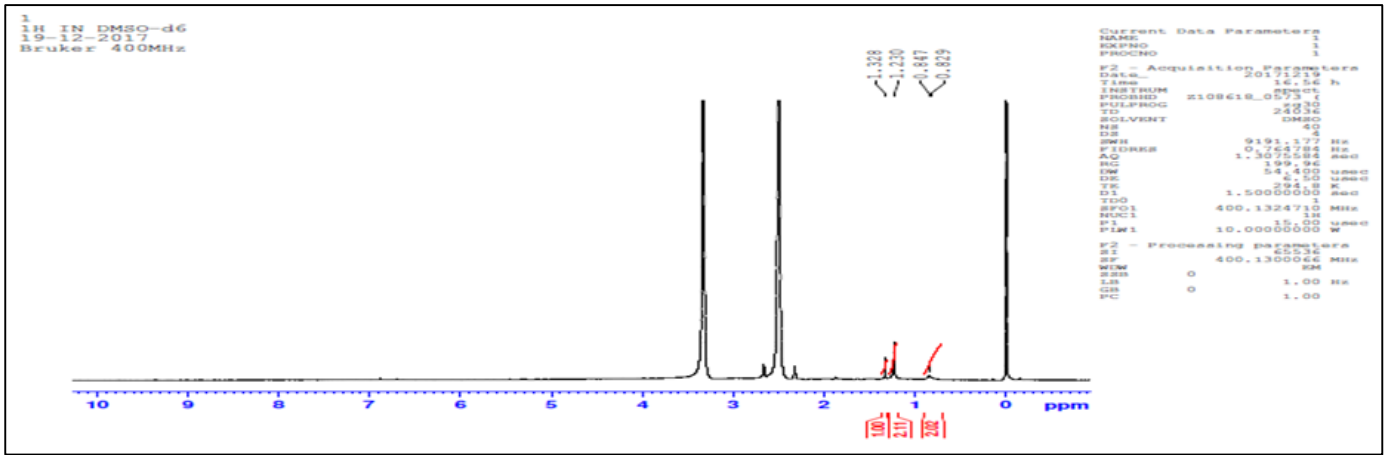
Fig 1: Characterization of main colored component of microbial extract of *Moringa oleifera* leaves.

FTIR Spectra of Moringa

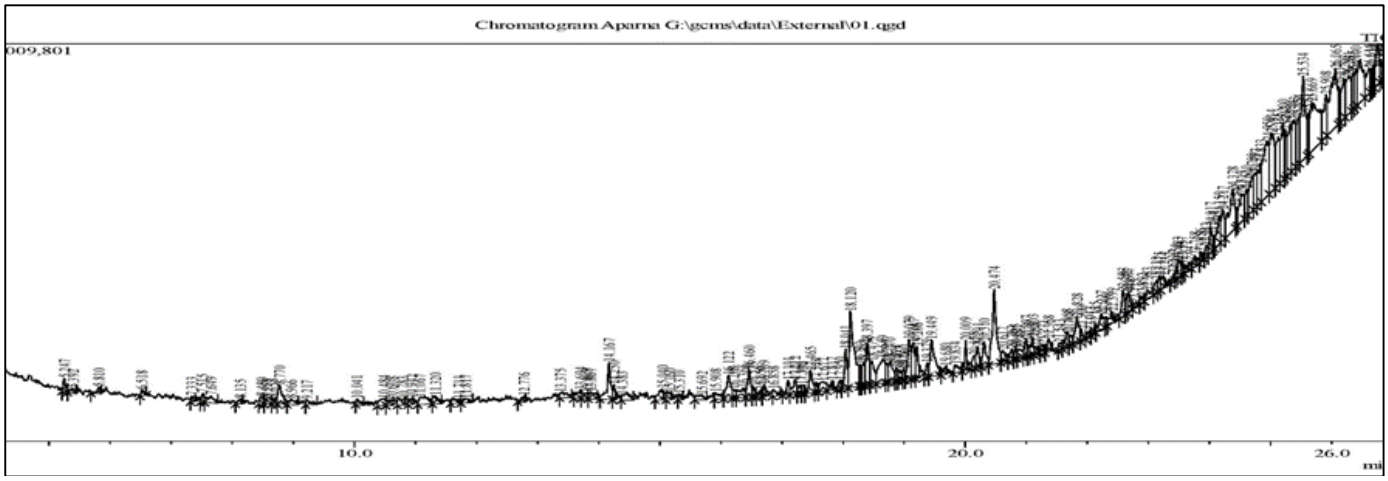


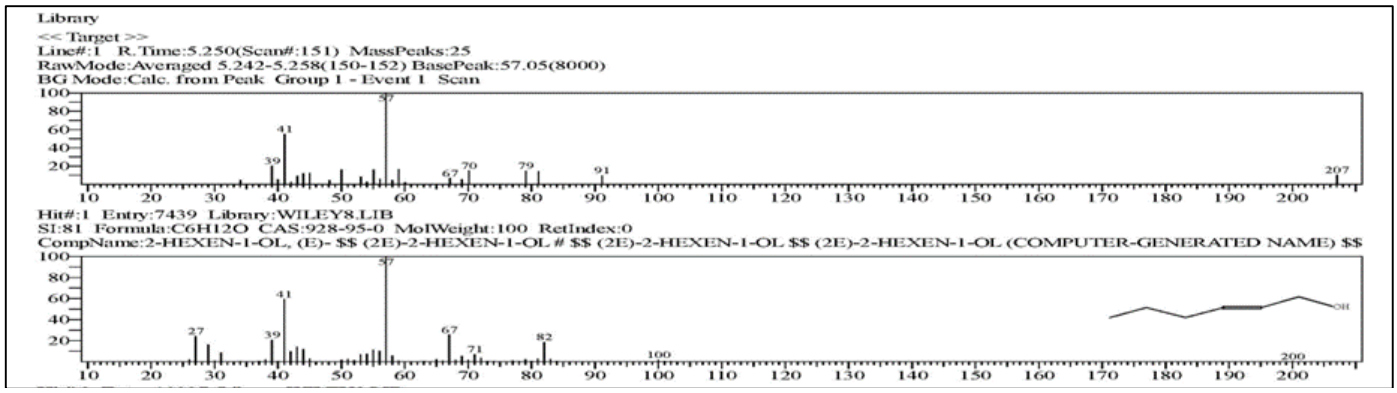
Absorption Peak value	Absorption range	Specific type of bond
3392.79	3500-3300	1^O amines (doublet), 2^O amines N-H stretch
3365.78	3500-3300	1^O amines (doublet), 2^O amines N-H stretch
2935.66	3000-2830	Alkanes C─H stretch
1716.65	1725-1703	C=O stretch, Carboxylic acids, Ketones
1597.06	1640-1550	Amides, 1^O and 2^O amines N-H stretch
1122.57	1300-1000	Alcohols, esters, ethers, -COOH, Anhydrides C-O stretch
617.22	800-600	Chloride C-Cl stretch

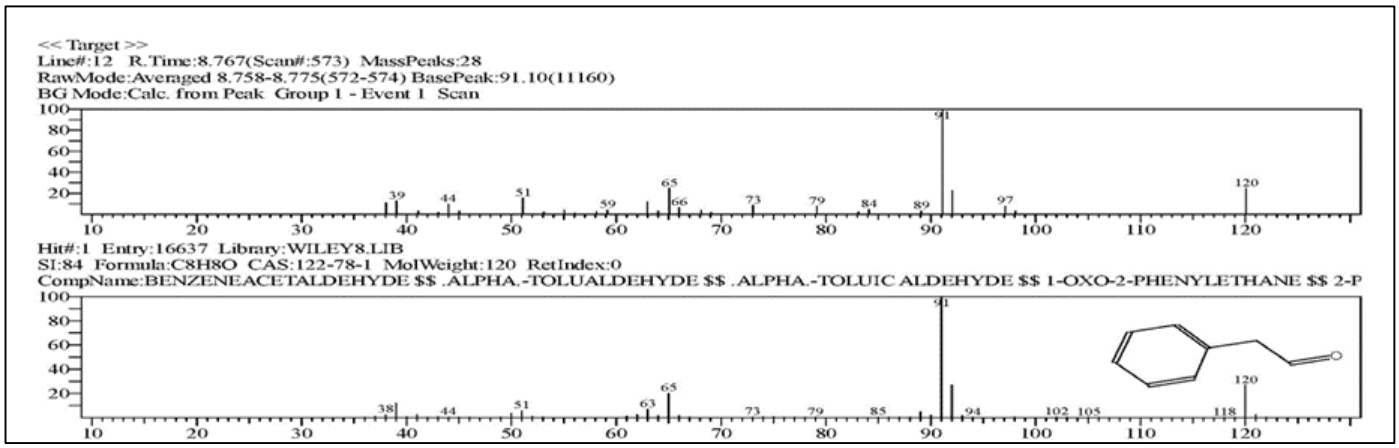
H-NMR-Spectra of Moringa

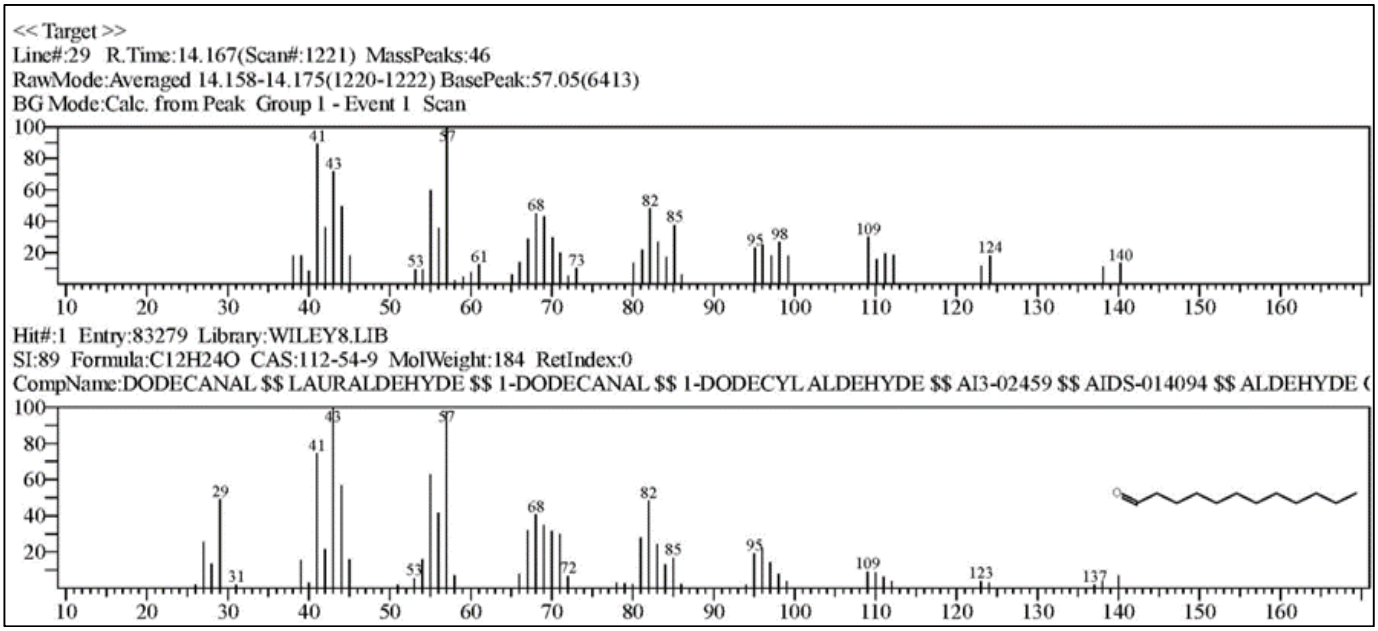


GC – MS Spectrum of Moringa





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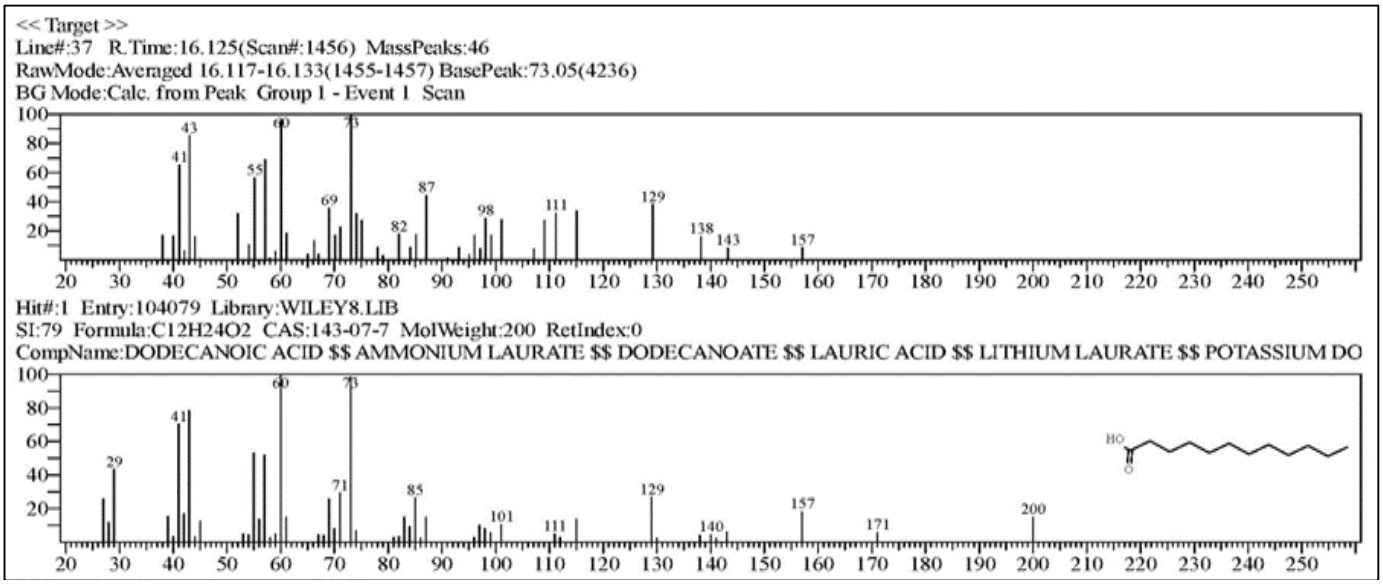
BG Mode:Calc. from Peak Group 1 - Event 1 Scan
Hit#:1 Entry:83279 Library:WILEY8.LIB
SI:89 Formula:C12H24O CAS:112-54-9 MolWeight:184 RetIndex:0
CompName:DODECANAL $$ LAURALDEHYDE $$ 1-DODECANAL $$ 1-DODECYL ALDEHYDE $$ AI3-02459 $$ AIDS-014094 $$ ALDEHYDE (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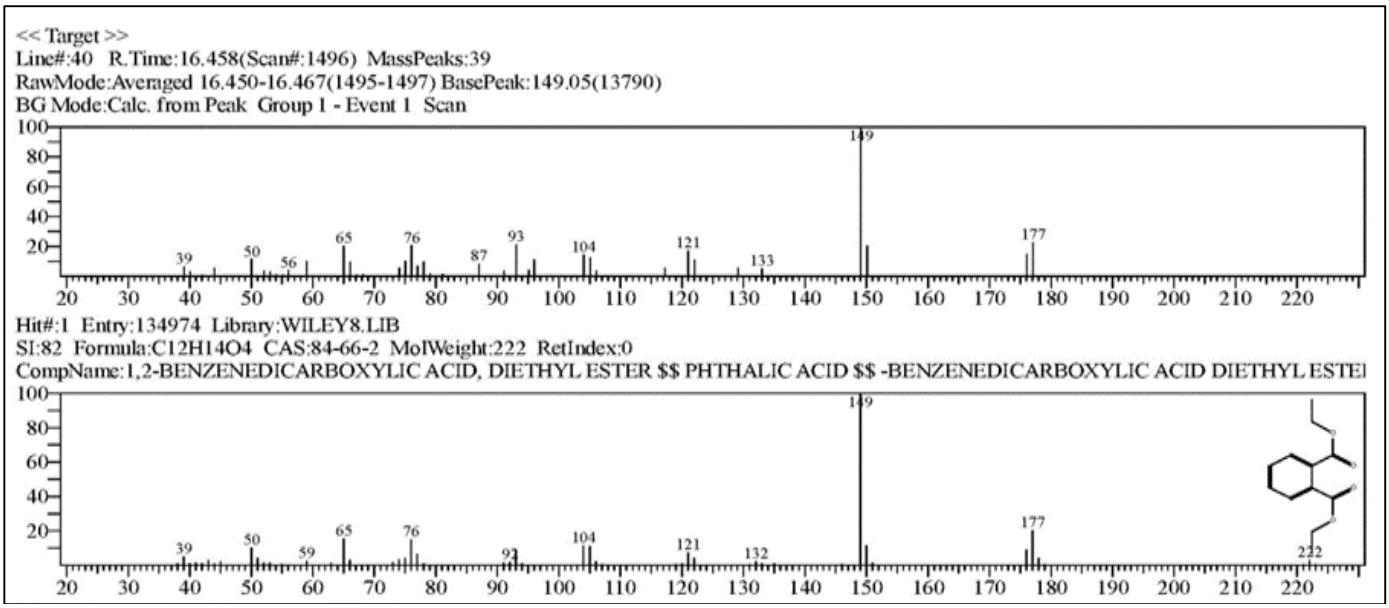
BG Mode:Calc. from Peak Group 1 - Event 1 Scan
Hit#:1 Entry:104079 Library:WILEY8.LIB
SI:79 Formula:C12H24O2 CAS:143-07-7 MolWeight:200 RetIndex:0
CompName:DODECANOIC ACID $$ AMMONIUM LAURATE $$ DODECANOATE $$ LAURIC ACID $$ LITHIUM LAURATE $$ POTASSIUM DO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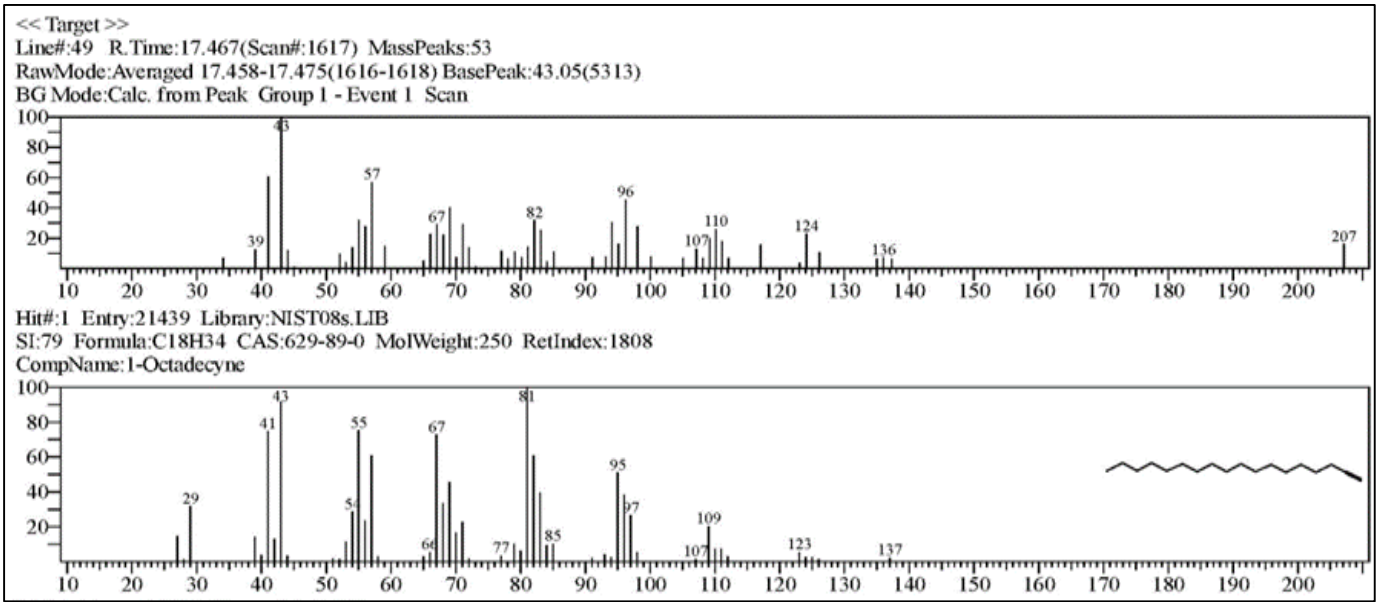
BG Mode:Calc. from Peak Group 1 - Event 1 Scan
Hit#:1 Entry:134974 Library:WILEY8.LIB
SI:82 Formula:C12H14O4 CAS:84-66-2 MolWeight:222 RetIndex:0
CompName:1,2-BENZENEDICARBOXYLIC ACID, DIETHYL ESTER $$ PHTHALIC ACID $$ -BENZENEDICARBOXYLIC ACID DIETHYL ESTEI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BG Mode:Calc. from Peak Group 1 - Event 1 Scan
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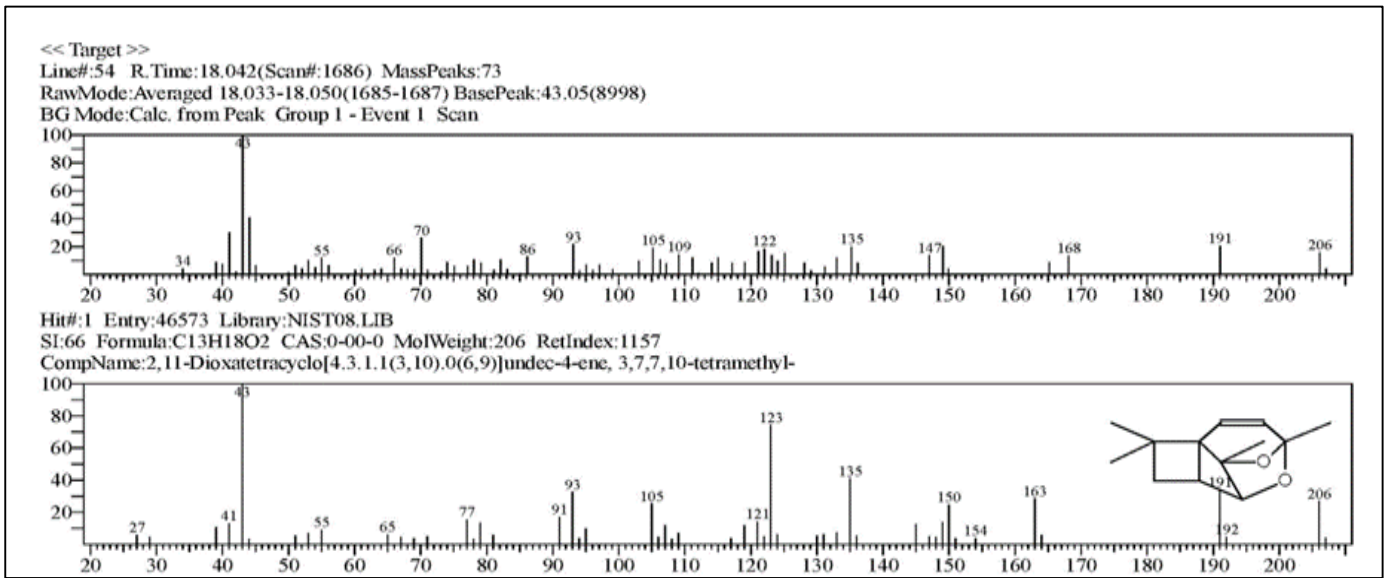
SI:79 Formula:C18H34 CAS:629-89-0 MolWeight:250 RetIndex:1808
CompName:1-Octadecyne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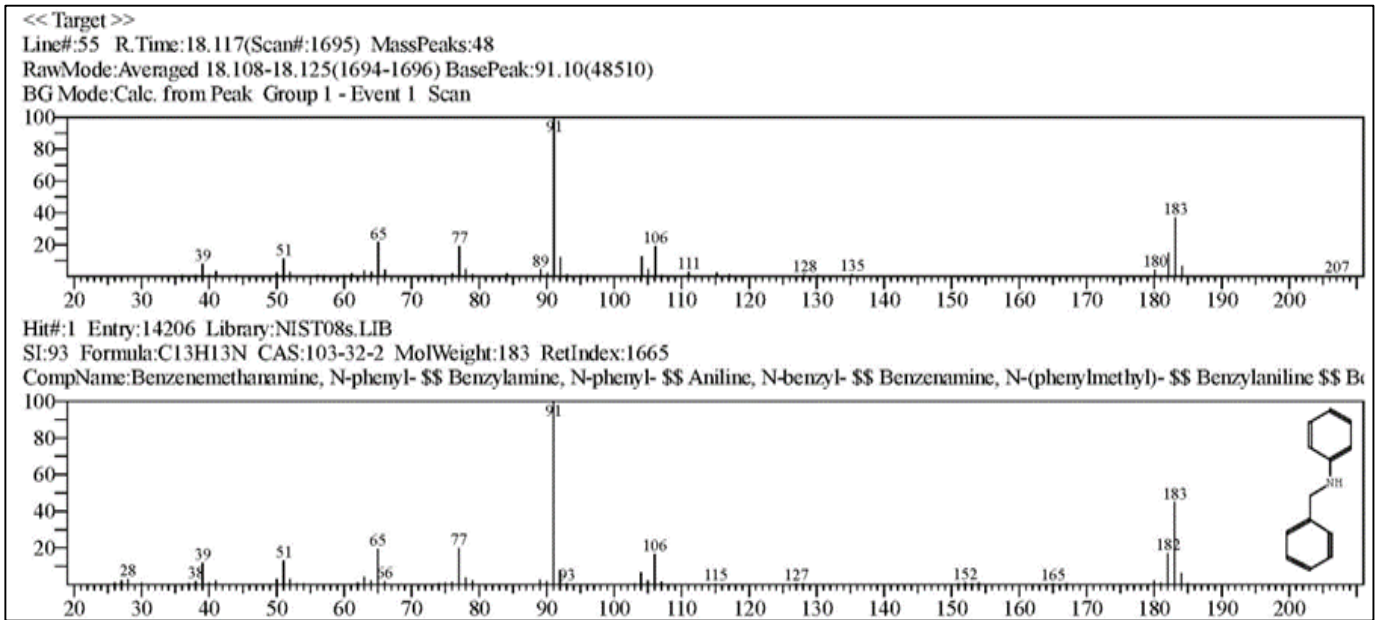
BG Mode:Calc. from Peak Group 1 - Event 1 Scan
Hit#:1 Entry:46573 Library:NIST08.LIB
SI:66 Formula:C13H18O2 CAS:0-00-0 MolWeight:206 RetIndex:1157
CompName:2,11-Dioxatetracyclo[4.3.1.1(3,10).0(6,9)]undec-4-ene, 3,7,7,10-tetramethyl-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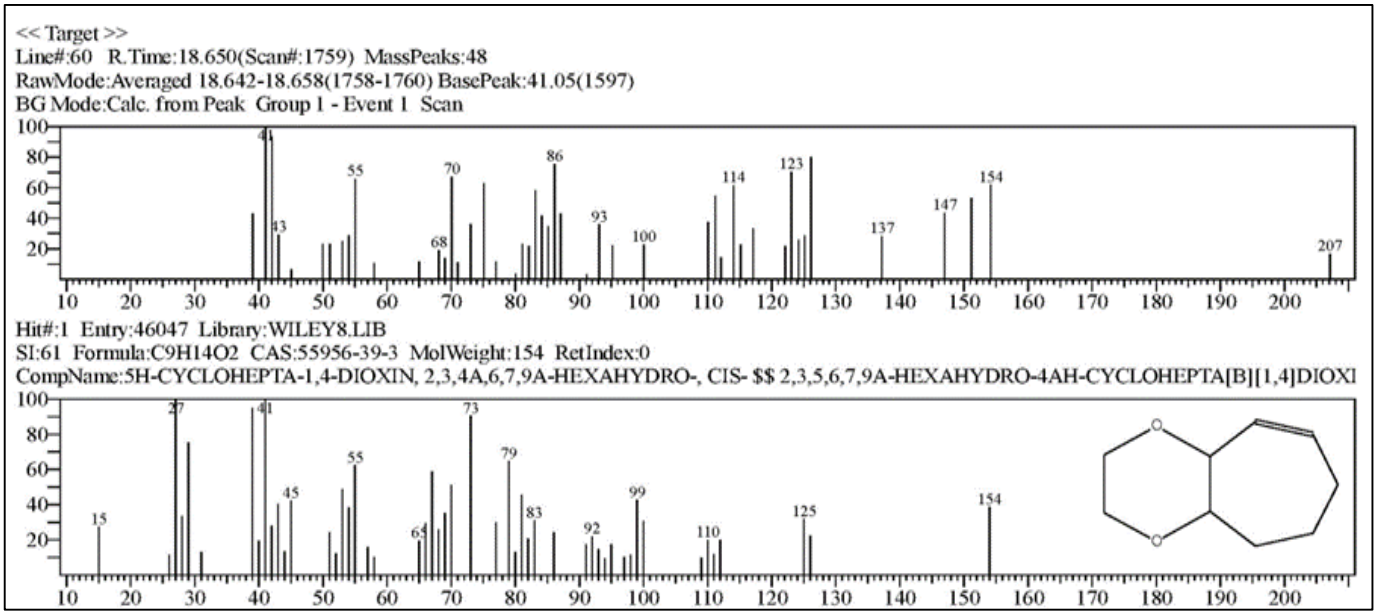
BG Mode:Calc. from Peak Group 1 - Event 1 Scan
Hit#:1 Entry:14206 Library:NIST08s.LIB
SI:93 Formula:C13H13N CAS:103-32-2 MolWeight:183 RetIndex:1665
CompName:Benzenemethanamine, N-phenyl- $$ Benzylamine, N-phenyl- $$ Aniline, N-benzyl- $$ Benzenamine, N-(phenylmethyl)- $$ Benzylaniline $$ B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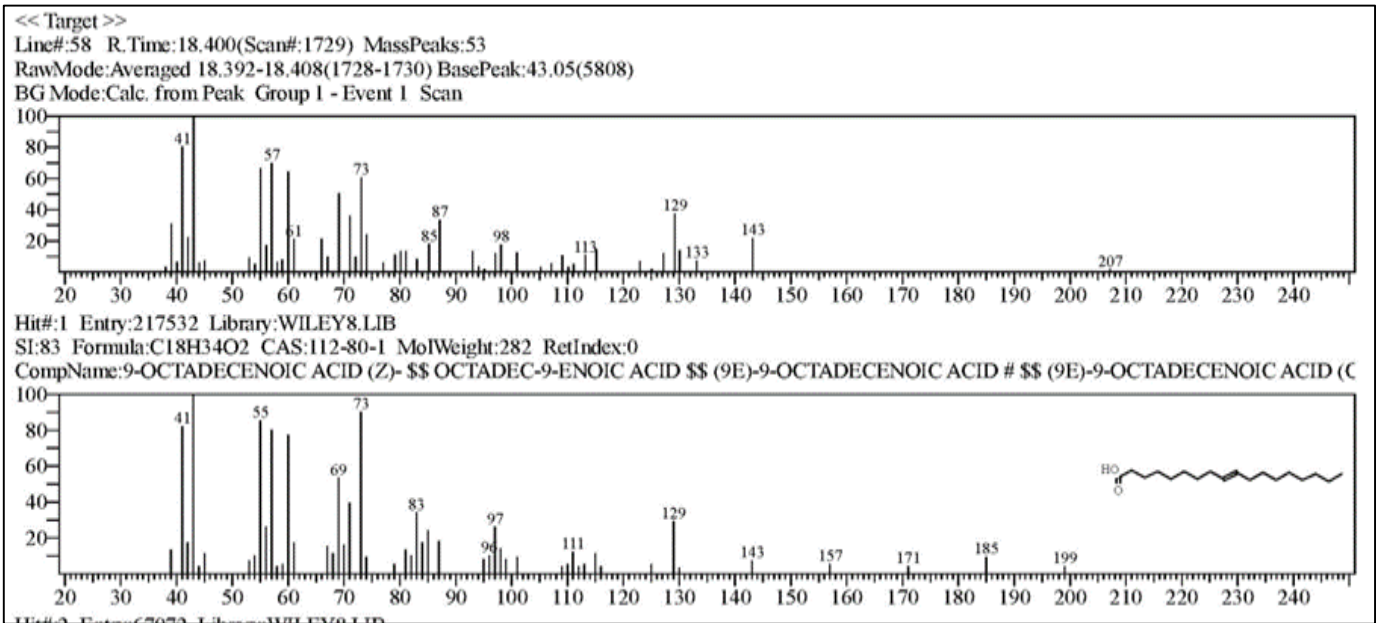
Hit#:1 Entry:46047 Library:WILEY8.LIB
SI:61 Formula:C9H14O2 CAS:55956-39-3 MolWeight:154 RetIndex:0
CompName:5H-CYCLOHEPTA-1,4-DIOXIN, 2,3,4A,6,7,9A-HEXAHYDRO-, CIS- $$ 2,3,5,6,7,9A-HEXAHYDRO-4AH-CYCLOHEPTA[B][1,4]DIOXI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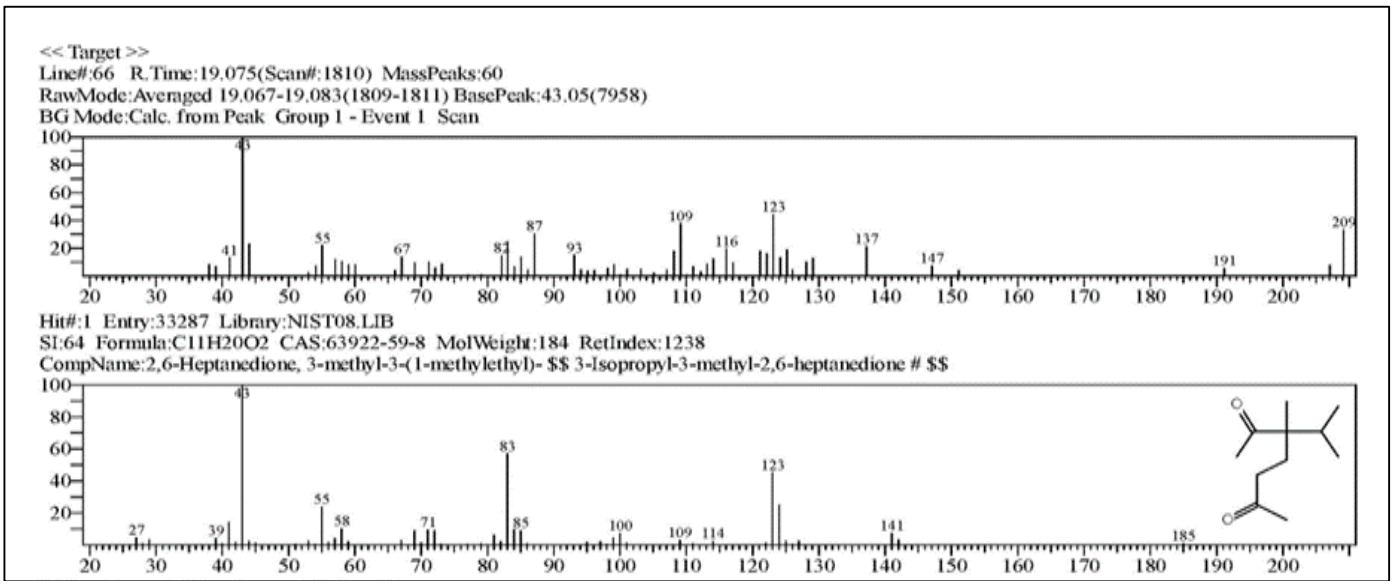
RawMode:Averaged 18.392-18.408(1728-1730) BasePeak:43.05(5808)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan
Hit#:1 Entry:217532 Library:WILEY8.LIB
SI:83 Formula:C18H34O2 CAS:112-80-1 MolWeight:282 RetIndex:0
CompName:9-OCTADECENOIC ACID (Z)- $$ OCTADEC-9-ENOIC ACID $$ (9E)-9-OCTADECENOIC ACID # $$ (9E)-9-OCTADECENOIC ACID (C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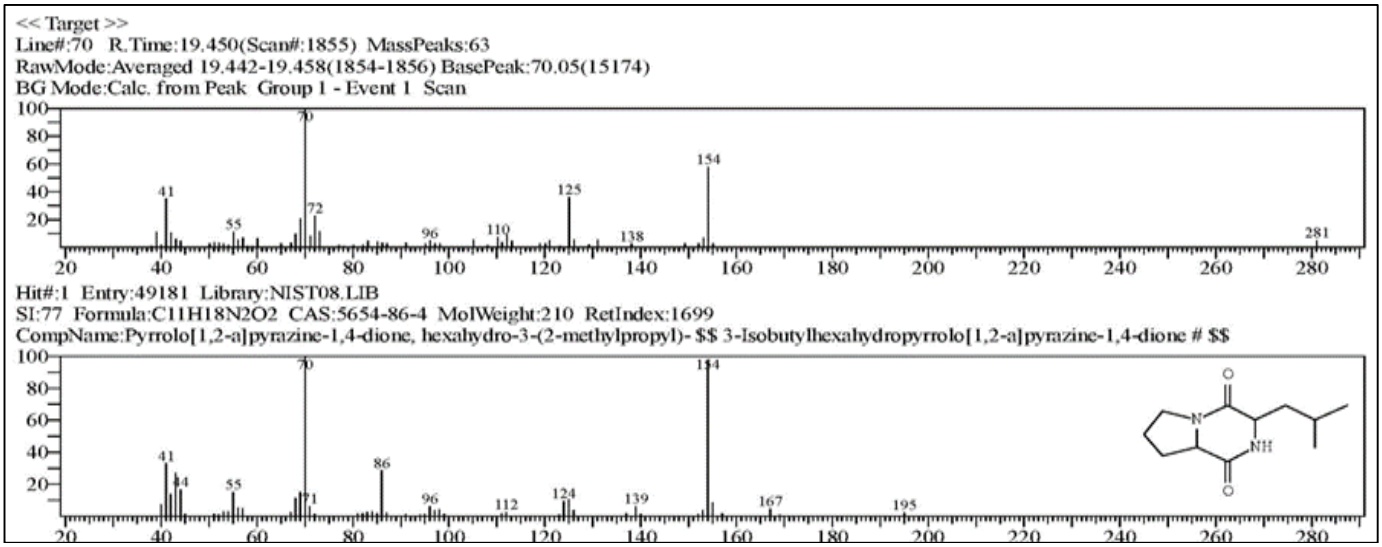
BG Mode:Calc. from Peak Group 1 - Event 1 Scan
Hit#:1 Entry:33287 Library:NIST08.LIB
SI:64 Formula:C11H20O2 CAS:63922-59-8 MolWeight:184 RetIndex:1238
CompName:2,6-Heptanedione, 3-methyl-3-(1-methylethyl)- $$ 3-Isopropyl-3-methyl-2,6-heptanedione # $$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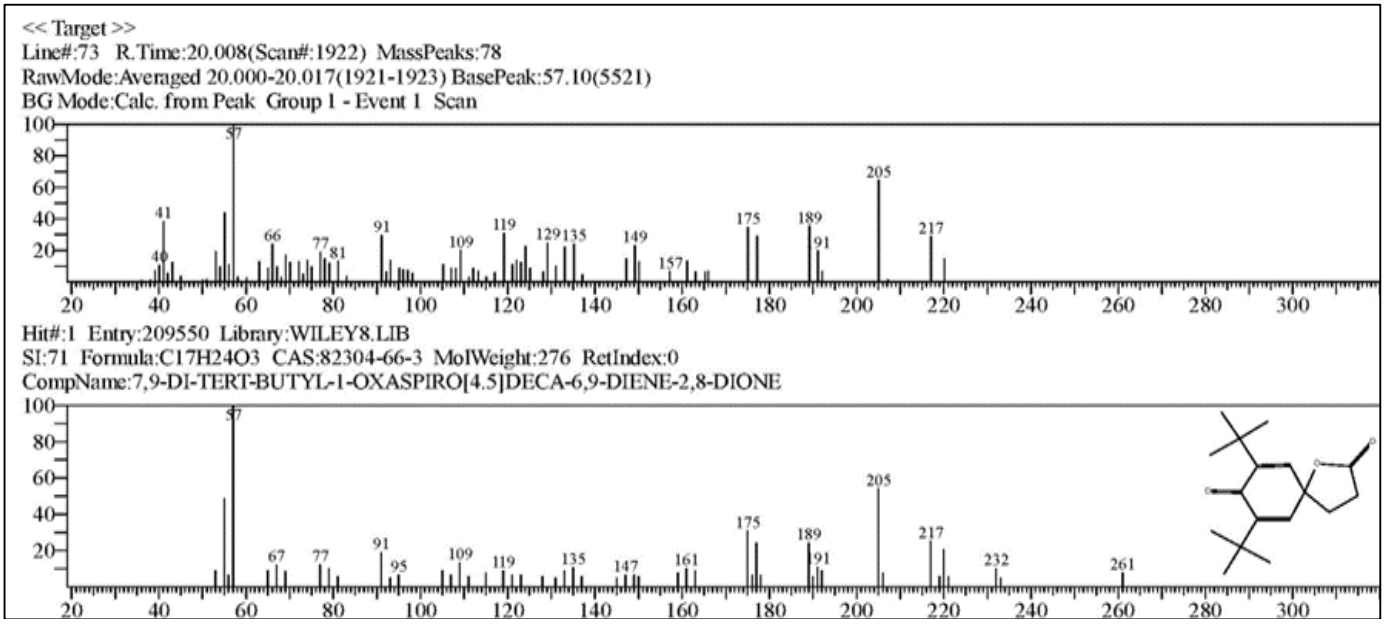
BG Mode:Calc. from Peak Group 1 - Event 1 Scan
Hit#:1 Entry:49181 Library:NIST08.LIB
SI:77 Formula:C11H18N2O2 CAS:5654-86-4 MolWeight:210 RetIndex:1699
CompName:Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)- $$ 3-Isobutylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione # $$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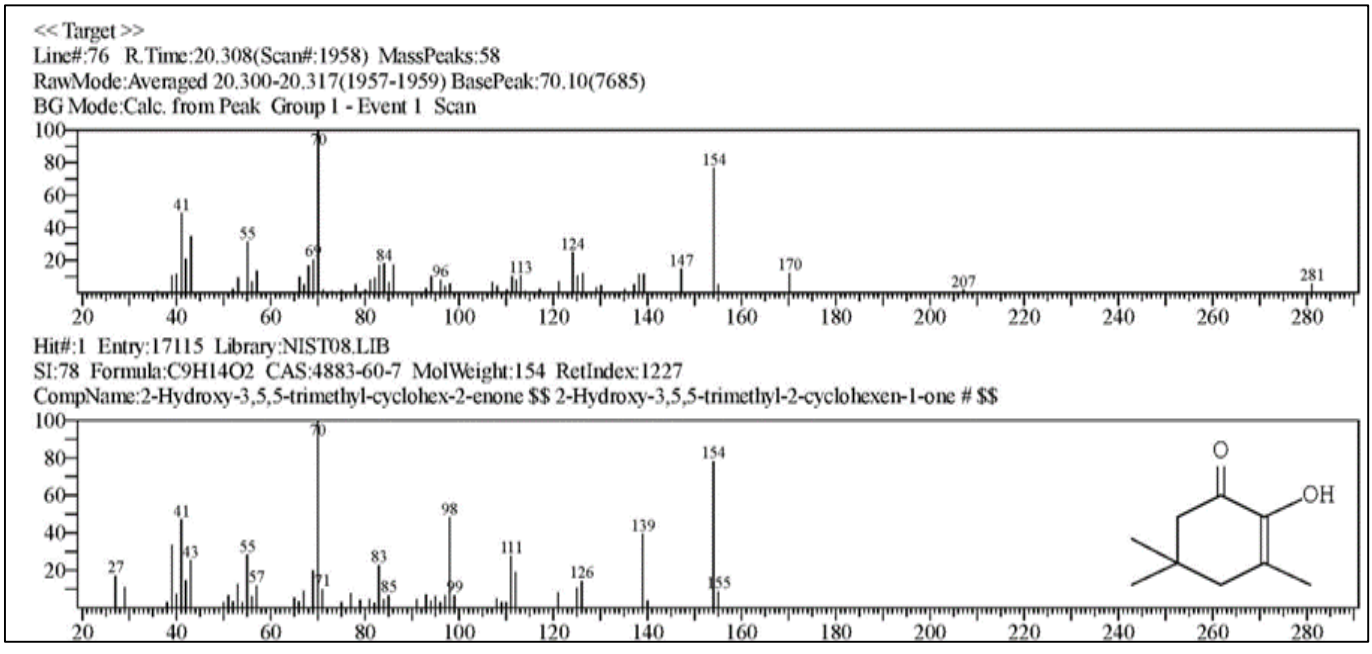
BG Mode:Calc. from Peak Group 1 - Event 1 Scan
Hit#:1 Entry:209550 Library:WILEY8.LIB
SI:71 Formula:C17H24O3 CAS:82304-66-3 MolWeight:276 RetIndex:0
CompName:7,9-DI-TERT-BUTYL-1-OXASPIRO[4.5]DECA-6,9-DIENE-2,8-DIONE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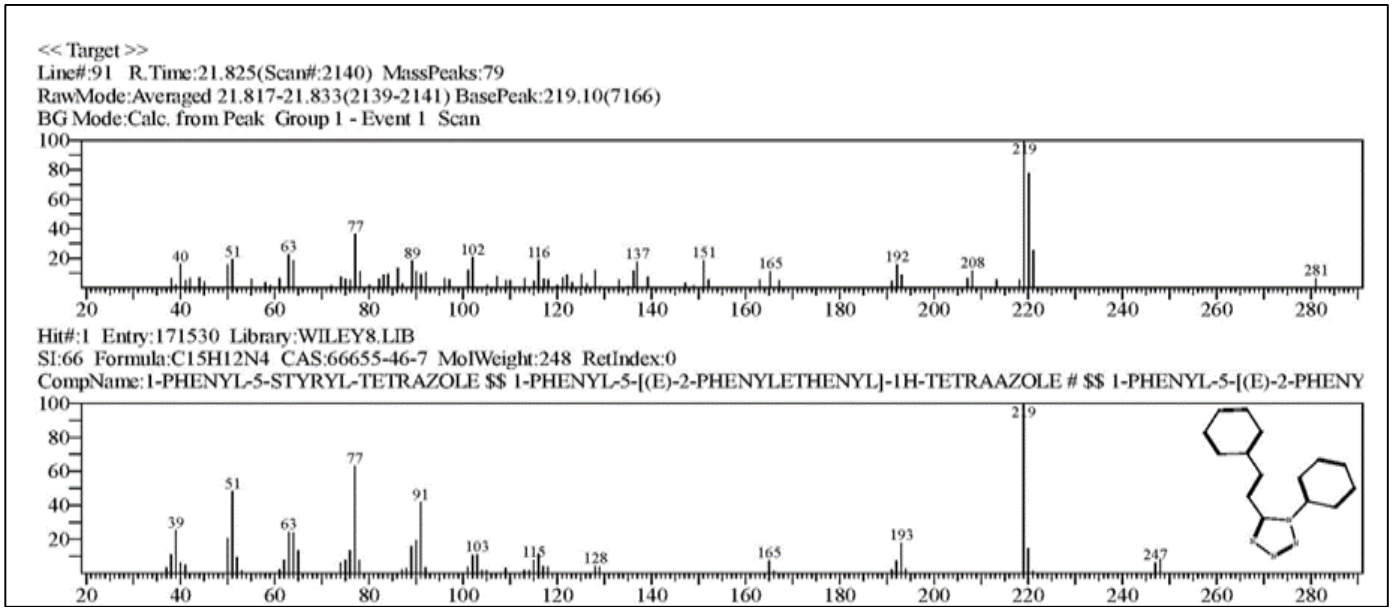
RawMode:Averaged 20.300-20.317(1957-1959) BasePeak:70.10(7685)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan
Hit#:1 Entry:17115 Library:NIST08.LIB
SI:78 Formula:C9H14O2 CAS:4883-60-7 MolWeight:154 RetIndex:1227
CompName:2-Hydroxy-3,5,5-trimethyl-cyclohex-2-enone $$ 2-Hydroxy-3,5,5-trimethyl-2-cyclohexen-1-one # $$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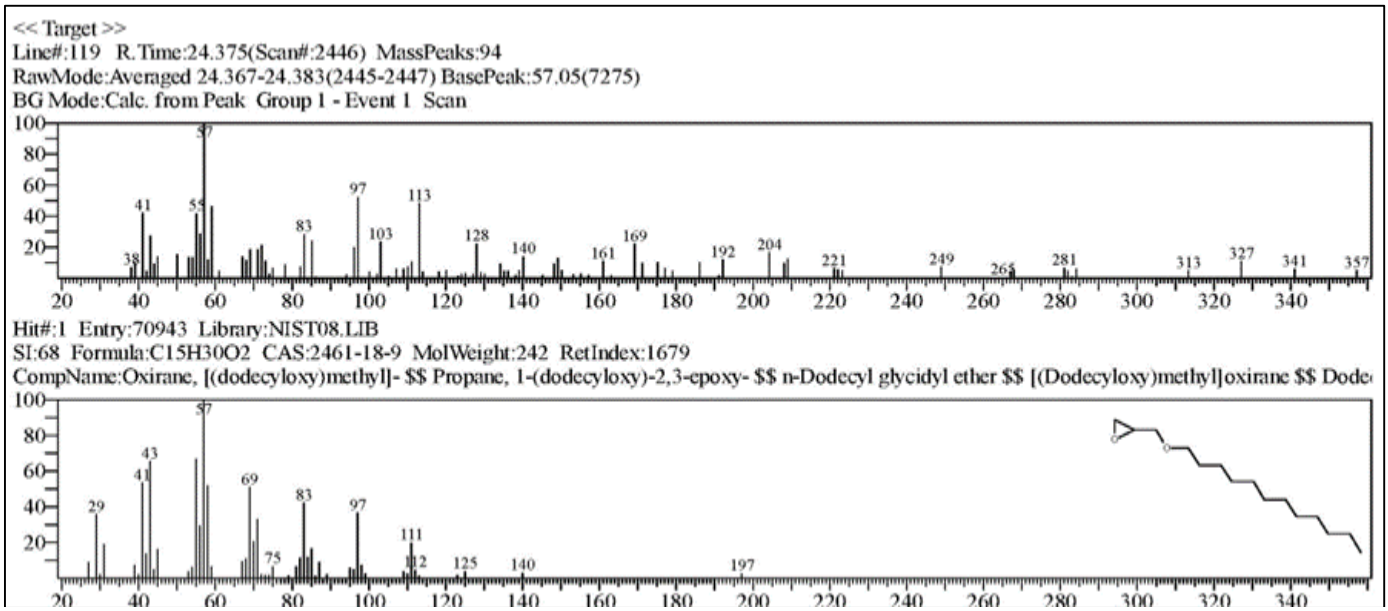
BG Mode:Calc. from Peak Group 1 - Event 1 Scan
Hit#:1 Entry:171530 Library:WILEY8.LIB
SI:66 Formula:C15H12N4 CAS:66655-46-7 MolWeight:248 RetIndex:0
CompName:1-PHENYL-5-STYRYL-TETRAZOLE $$ 1-PHENYL-5-[(E)-2-PHENYLETHENYL]-1H-TETRAAZOLE # $$ 1-PHENYL-5-[(E)-2-PHENY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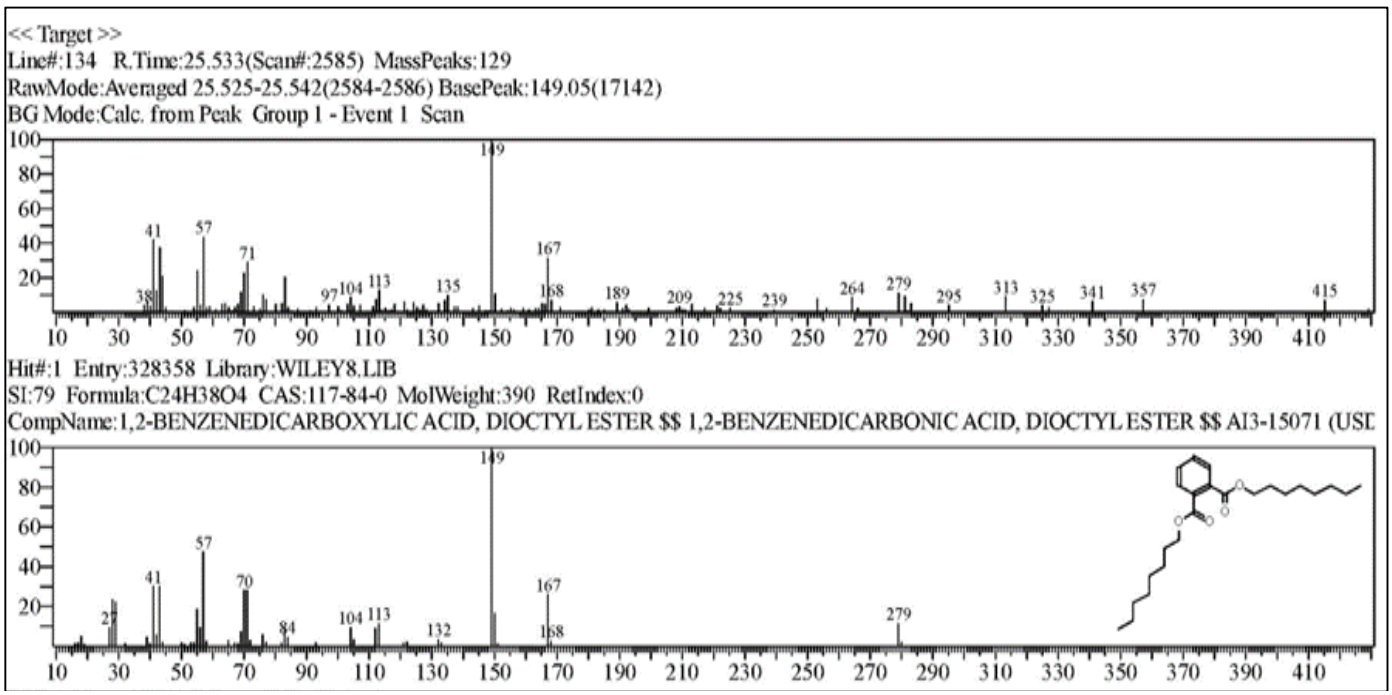
RawMode:Averaged 24.367-24.383(2445-2447) BasePeak:57.05(7275)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan
Hit#:1 Entry:70943 Library:NIST08.LIB
SI:68 Formula:C15H30O2 CAS:2461-18-9 MolWeight:242 RetIndex:1679
CompName:Oxirane, [(dodecyloxy)methyl]- $$ Propane, 1-(dodecyloxy)-2,3-epoxy- $$ n-Dodecyl glycidyl ether $$ [(Dodecyloxy)methyl]oxirane $$ Dode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BG Mode:Calc. from Peak Group 1 - Event 1 Scan
Hit#:1 Entry:328358 Library:WILEY8.LIB
SI:79 Formula:C24H38O4 CAS:117-84-0 MolWeight:390 RetIndex:0
CompName:1,2-BENZENEDICARBOXYLIC ACID, DIOCTYL ESTER $$ 1,2-BENZENEDICARBONIC ACID, DIOCTYL ESTER $$ AI3-15071 (USI

Major phytochemical compounds identified in microbial extract of *Moringa oleifera* leaf

S.no	RT (min)	Name of the compound	Molecular Formula	Molecular Weight	Peak Area %	Pharmacological purpose
1	5.247	2-Hexen-1-ol (E)	$C_6H_{12}O$	100	0.28	Flavouring agent
2	8.770	Benzeneacetaldehyde	C_8H_8O	120	0.69	Flavouring agent
3	14.167	Dodecanal	$C_{12}H_{24}O$	184	1.21	Flavouring agent, Fragrances
4	16.125	Dodecanoic Acid	$C_{12}H_{24}O_2$	200	0.94	Flavouring agent, Herbicide, Insecticide, Surfactants
5	16.460	1,2-Benzenedicarboxylic acid diethyl ester	$C_{12}H_{14}O_4$	222	0.80	1. Flavouring agent, Phthalates, Adhesives and sealant chemicals, 2. Odor agents, and 3. Plasticizers
6	17.465	1-Octadecyne	$C_{18}H_{34}$	250	0.75	No activity reported
7	18.041	2,11-Dioxatetracyclo [4,3,1,1(3,10),0(6,9)]undec-4-ene,	$C_{13}H_{18}O_2$	206	0.97	No activity reported
8	18.120	Benzenemethanamine, N-phenyl	$C_{13}H_{13}N$	183	3.45	No acitivity reported
9	18.397	9-Octadecanoic Acid	$C_{18}H_{34}O_2$	282	1.22	Therapeutic drug, Antibacterial activity
10	18.649	5H-Cyclohepta-1,4-dioxin	$C_9H_{14}O_2$	154	1.41	No activity reported
11	19.079	2,6-Heptanedione	$C_{11}H_{20}O_2$	184	1.17	No activity reported
12	19.449 20.474	Pyrrolo (1,2-a)pyrazine-1,4-dione	$C_{11}H_{18}N_2O_2$	210	1.58 2.91	Antibiotic agent, Anticandidal agent
13	20.009	7,9-di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276	0.51	Ophthalmic drug activity
14	20.310	2-Hydroxy-3,5,5-trimethyl-cyclohex-2-enone	$C_9H_{14}O_2$	154	0.76	Antityrosinase activity
15	21.828	1-Phenyl-5-styryl-tetrazole	$C_{15}H_{12}N_4$	248	0.92	Anti-inflammatory, Anti-microbial agents
16	24.375	Oxirane, [(dodecyloxy)methyl]-	$C_{15}H_{30}O_2$	242	2.13	Surfactants
17	25.534	1,2-Benzenedicarboxylic acid	$C_{24}H_{38}O_4$	390	3.31	1. Plasticizer, Paints and coatings, Food packaging, Food Contaminant

Now a day the study of the organic compounds from plants and their activity has increased. Gas Chromatography – Mass Spectrometry (GC - MS) is a valuable tool for reliable identification of bioactive compounds (Johnson *et al*., 2011) [6]. In the present study, 150 compounds have been identified from the microbial extract of leaves of *M. oleifera* by GC - MS analysis. The most abundant 17 components found in the leaves were 2-Hexen-1-ol (E), Benzene acetaldehyde, Dodecanal, Dodecanoic Acid, 1,2-Benzenedicarboxylic acid diethyl ester, 1-Octadecyne, 2,11-Dioxatetracyclo[4,3,1,1 (3,10),0 (6,9)] undec-4-ene, Benzenemethanamine, N-phenyl, 9-Octadecanoic Acid, 5H-Cyclohepta-1,4-dioxin, 2,6-Heptanedione, Pyrrolo (1,2-a) pyrazine-1,4-dione, 7,9-di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione, 2-Hydroxy-3,5,5-trimethyl-cyclohex-2-enone, 1-Phenyl-5-styryl-tetrazole, Oxirane, [(dodecyloxy) methyl]-, and 1,2-Benzenedicarboxylic acid.

5. Conclusion

The most significant outcome of this study was the production of red pigment from *P. purpurogenum* under various nutritional conditions. It could be seen that *P. purpurogenum* responded by producing high concentrations of pigment of *M. oleifera* leaves. The results of the optimization, spectroscopic characterization indicates that the isolated pigment having different phytochemicals were produced having flavouring agents, antibacterial activity. The structural elucidation from GCMS shows that structure of the main pigment constituent in the pigment. To the best of our knowledge, this is the first study to report red pigment production by *P. purpurogenum* from leaves.

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7. References

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