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Dark goldenrod pigment production by *p. purpurogenum* using *Trigonella. foenum-graecum* leaves and characterization and identification of phytochemicals by analytical techniques

Sri Vani Aparna. Sriram and Dr Dhurjeti Sarvamangala

Abstract
In this study, the bioactive components of *Trigonella foenum-graecum* leaves have been evaluated using techniques like UV-Vis, FTIR, Proton-NMR spectroscopy and Gas Chromatography-Mass Spectrometry. The chemical compositions of the microbial extract of *Trigonella foenum-graecum* leaves were investigated from Gas Chromatography–Mass Spectrometry, the spectra of the compounds found in the extract were matched with the National Institute of Standards and Technology (NIST) library. GC/MS analysis of the microbial extract of *Trigonella foenum-graecum* leaves revealed the existence of The results of this study offers a platform for using *Trigonella foenum-graecum* leaves as the herbal alternative for the current synthetic antimicrobial agents.

Keywords: Ultraviolet spectroscopy, antimicrobial agents, NIST library.

1. Introduction
Nowadays, traditional herbal medicines are gaining popularity as the primary health care needs, as an alternative and complementary therapies [1]. These plants can be used as a wide range of biological and pharmacological activities such as anti-cancer, anti-inflammatory, diuretic, oxytocic, laxative, antispasmodic, antihypertensive, anti-diabetic, and anti-microbial functions. The secondary metabolites of plants provide humans with numerous biological active products which have been used extensively as drugs, foods, additives, flavors, insecticides, colorants, fragrances and chemicals [2]. The leaves of the plant have been used as a traditional remedy for numerous conditions including gastrointestinal disorders, gout, wound healing and inflammation, hyperlipidemia and diabetes [3]. The antihyperglycemic effects of fenugreek leaves and its subfractions are demonstrated in diabetic rats [4] dogs [5] and humans. The leaves also show beneficial effects in hypolipidemic subjects [6] and in cancer [7]. Supplementation of leaves in the diet enhances the antioxidant potential in control and diabetic rats [8]. It has been reported to have restorative and nutritive properties and to stimulate digestive processes, useful in healing of ulcers in digestive tract [9]. Bioactive compounds isolated from fenugreek leaves include saponins, amino acids, flavonoids, coumarins, mucilaginous fibers, and other vitamins and minerals [10, 3]. Flavonoids have remarkable biological activities, including inhibitory effects on enzymes, a modulatory effect on some cell types, protection against allergies, antibacterial, antifungal, antiviral, antimalarial, antioxidant, anti-inflammatory and anticarcinogenic properties [11]. Much of the hypoglycemia effect of fenugreek leaves in clinical studies is likely due to the inhibitory effects of mucilaginous fibers on glucose absorption [12, 13] Hence it was planned to study the phytochemicals present in the microbial of *Trigonella foenum-graecum* leaves by the analytical tools to validate its use in textiles.

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 *Trigonella. foenum-graecum* were collected in fresh polythene bags from Kakinada, East Godavari district, A.P.

3. Methodology
3.1 Production of microbial pigments from *Trigonella foenum-graecum*
The leaves were initially washed in tap water, then with distilled water to remove soil and other contaminants. They were weighed and ground into a paste and 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 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 were slowly removed on evaporation leaving the solid concentrate. The concentrate was cooled immediately. The crude extract obtained was allowed for 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[5].

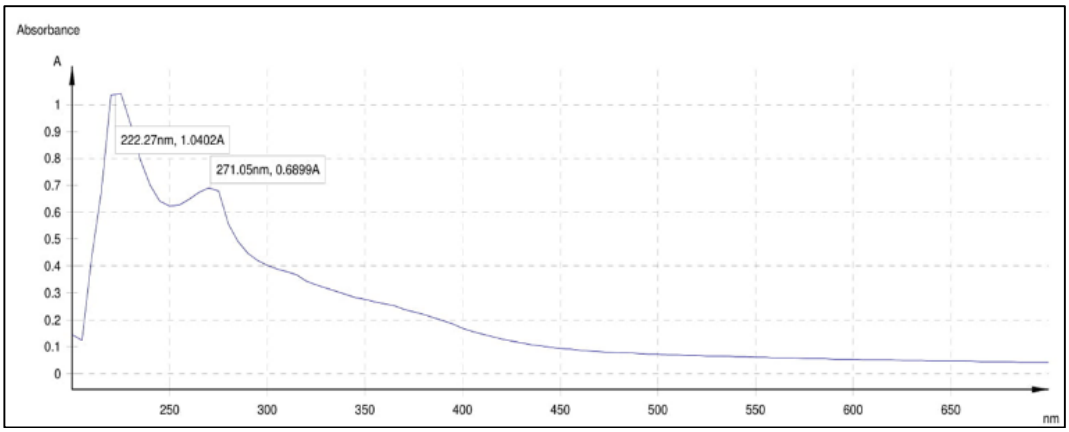
3.3. Fourier Transform Infra-Red (FT-IR) spectroscopy
The Fourier transform infrared (FT-IR) spectrum was recorded on a Bruker FT-IR spectrophotometer and the spectral range was 4000 – 500 cm-1**[6].** The dried powder of the red pigment was scanned by a Shimadzu FT-IR 8000 spectrophotometer in the 4000–400 cm−1 range using the KBr method at 27 °C.

3.4. Nuclear Magnetic Resonance Spectroscopy
The purified pigment was dissolved with dimethylsulphoxide (DMSO d6) and the sample was injected into a nuclear magnetic resonance (NMR) spectrometer (Bruker 400 MHz)[7].

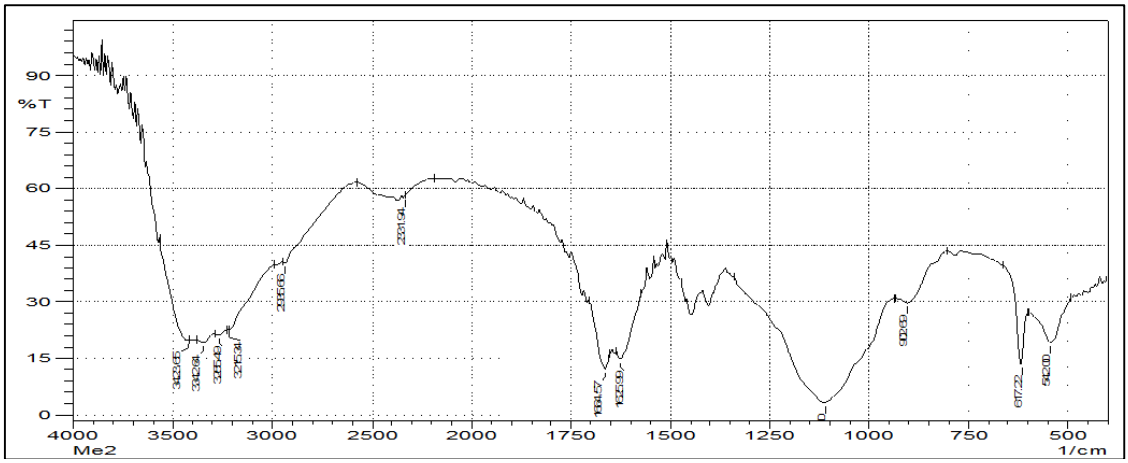
3.5. Gas Chromatography-Mass Spectrometry
In the current study, the microbial extract of *Trigonella. foenum-graecum* was screened for the presence of phytochemical compounds by qualitative test procedures followed by GC-MS analysis of novel compounds. This study was facilitated by using Gas Chromatography-Mass Spectrometry. The mass spectra of compounds in the microbial extract were matched with NIST (National Institute of Standards and Technology) and WILEY library.

4. Results and Discussion
The pigment produced from fermentation of under the optimal conditions were p^H 5.5, temperature 37ºC and production time 8 days. The highest production of *Trigonella foenum-graecum* L. pigments is 1.9% i.e., 19.8g/L and the maximum absorbance at 222 nm (1.0402). The produced pigment was characterized using different techniques like UV Visible Spectroscopy, FTIR and 1H NMR and GC-MS techniques.
Figure no 1:- Characterization of the main colored component of the microbial extract of *Trigonella foenum-graecum* leaves.

UV-Visible Spectra

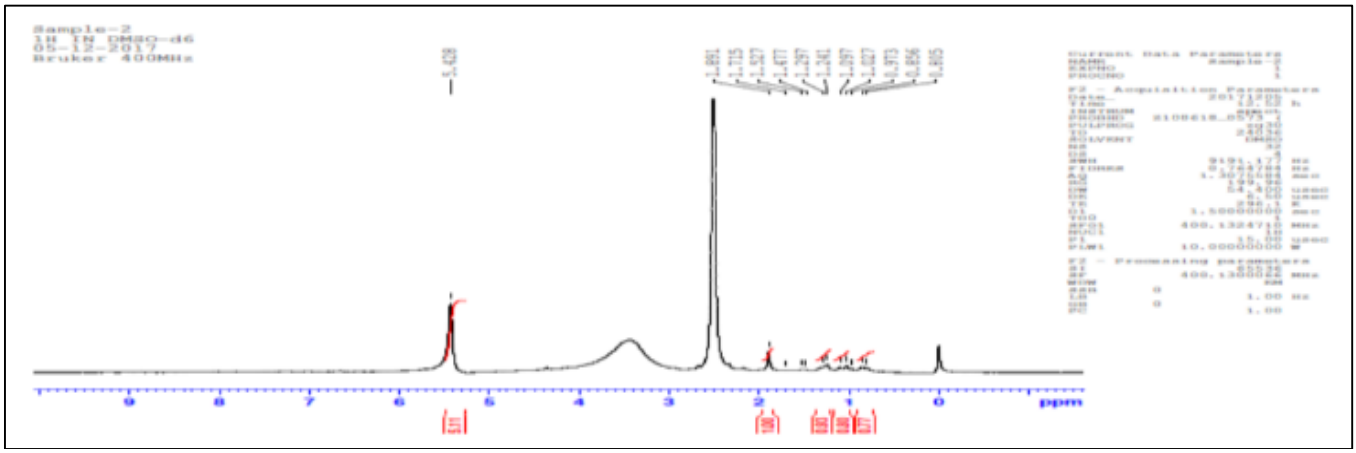


FTIR Spectra

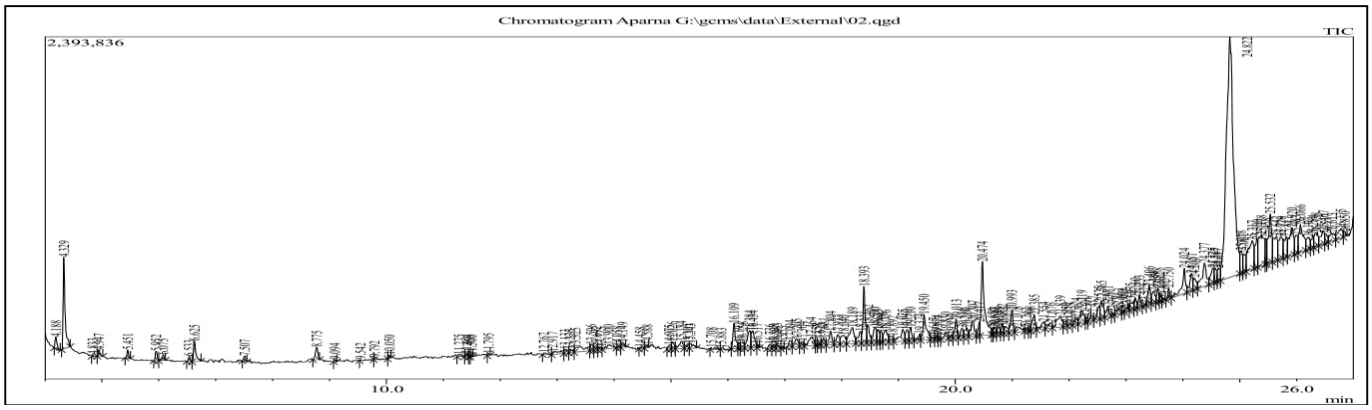


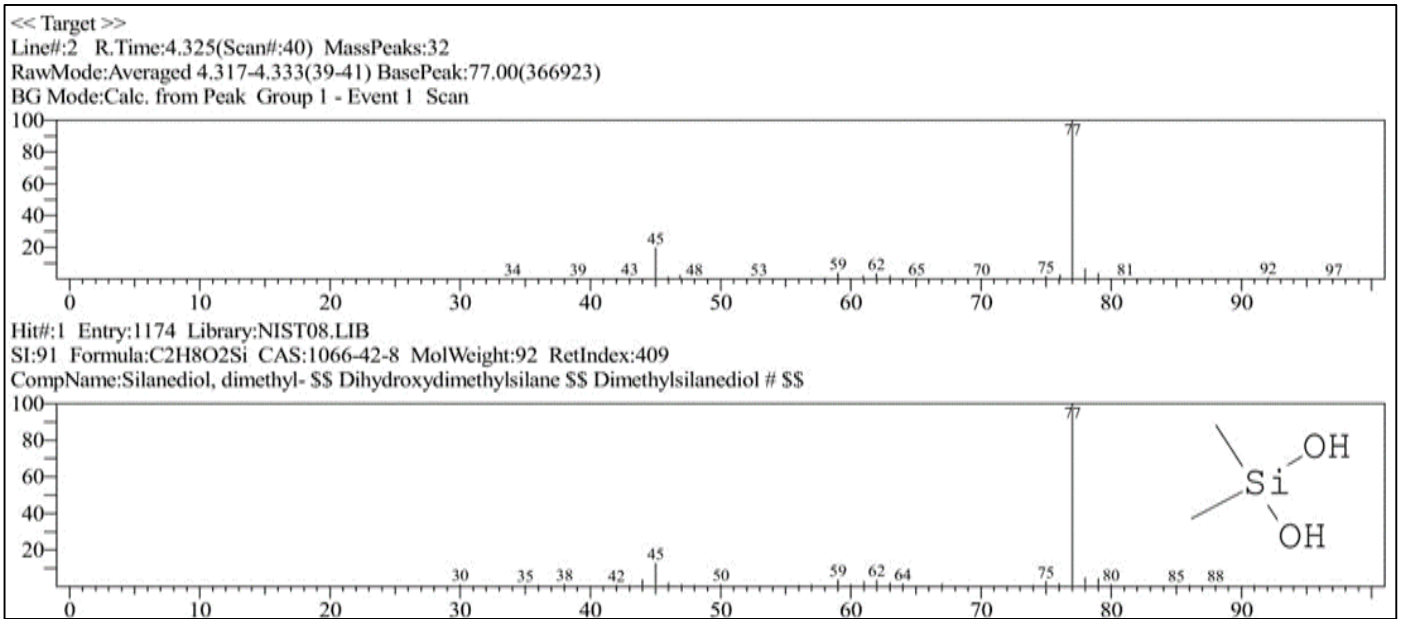
Absorption Peak value	Absorption range	Specific type of bond
3423.65	3500-3300	1^{O} amines (doublet), 2^{O} amines N-H stretch
3342.64	3500-3300	1^{O} amines (doublet), 2^{O} amines N-H stretch
2935.66	3000-2830	Alkanes C─H stretch
1664.57	1670-1640	Amides C=O stretch (Amide II band)
1625.99	1640-1550	Amides, 1^{O} and 2^{O} amines N-H stretch
1125.34	1300-1000	Alcohols, esters, ethers, -COOH,
		Anhydrides C-O stretch
902.69	1000-650	Alkenes C-H out of plane bend
617.22	800-600	Chloride C-Cl stretch

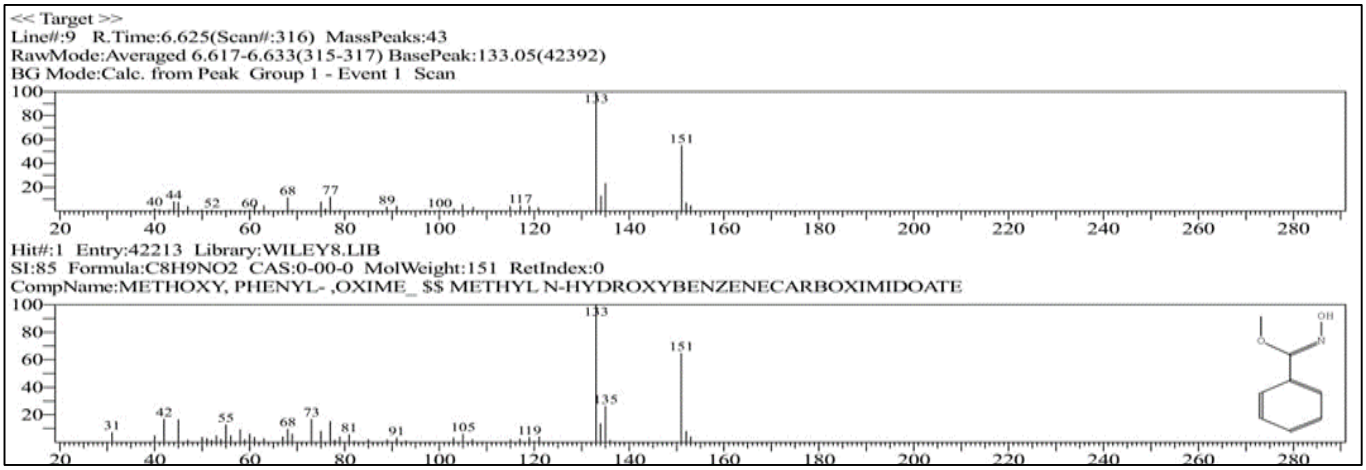
H-NMR Spectra



GC-MS Spectrum



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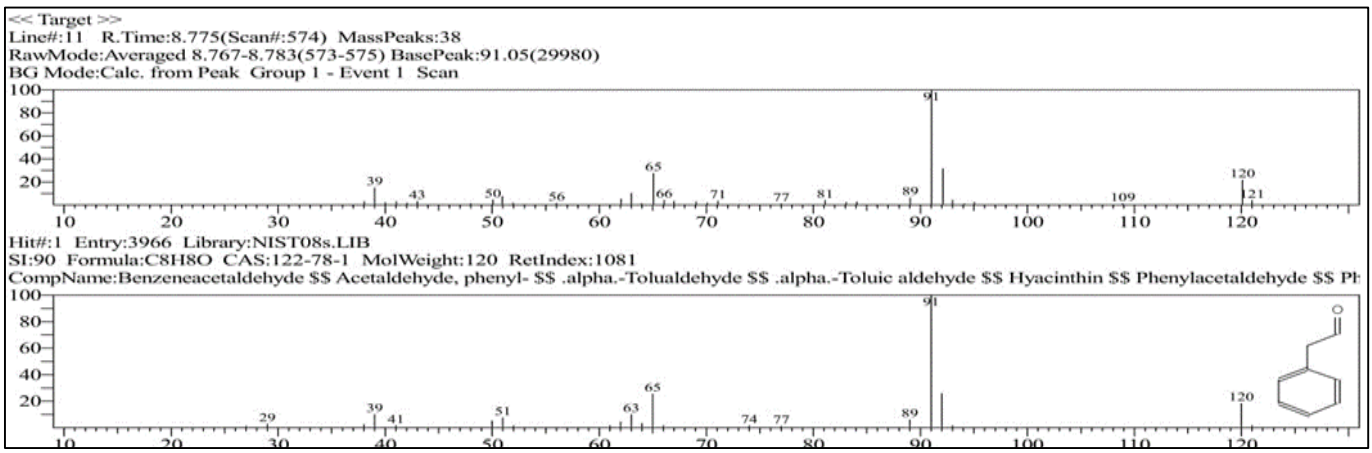
RawMode:Averaged 6.617-6.633(315-317) BasePeak:133.05(42392)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan
Hit#:1 Entry:42213 Library:WILEY8.LIB
SI:85 Formula:C8H9NO2 CAS:0-00-0 MolWeight:151 RetIndex:0
CompName:METHOXY, PHENYL- ,OXIME_ $$ METHYL N-HYDROXYBENZENECARBOXIMIDOATE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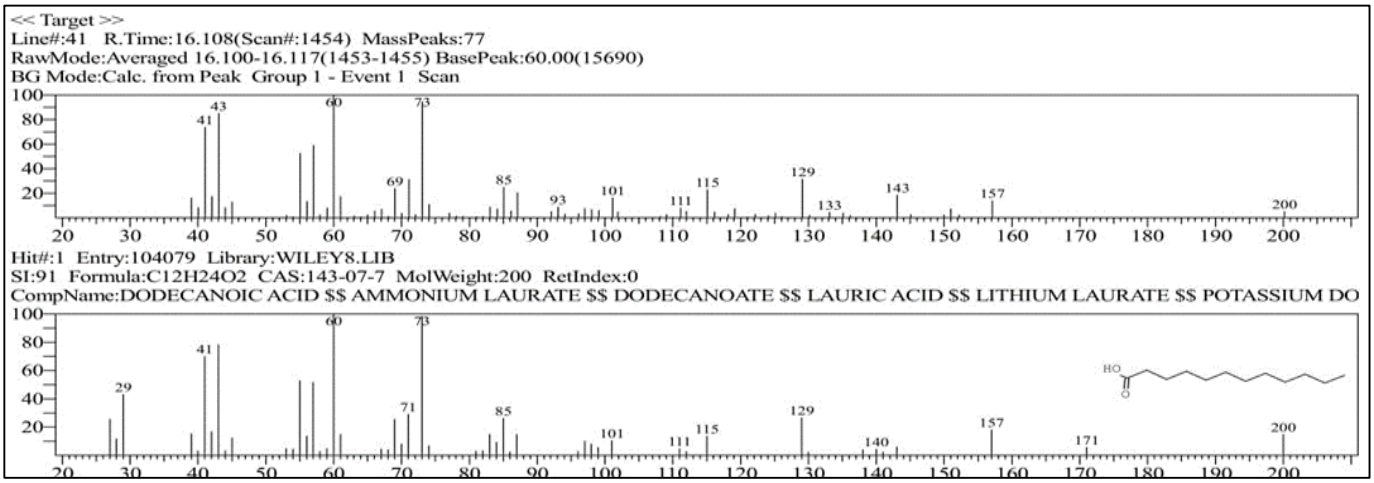
RawMode:Averaged 8.767-8.783(573-575) BasePeak:91.05(29980)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan
Hit#:1 Entry:3966 Library:NIST08s.LIB
SI:90 Formula:C8H8O CAS:122-78-1 MolWeight:120 RetIndex:1081
CompName:Benzeneacetaldehyde $$ Acetaldehyde, phenyl- $$ .alpha.-Tolualdehyde $$ .alpha.-Toluic aldehyde $$ Hyacinthin $$ Phenylacetaldehyde $$ Ph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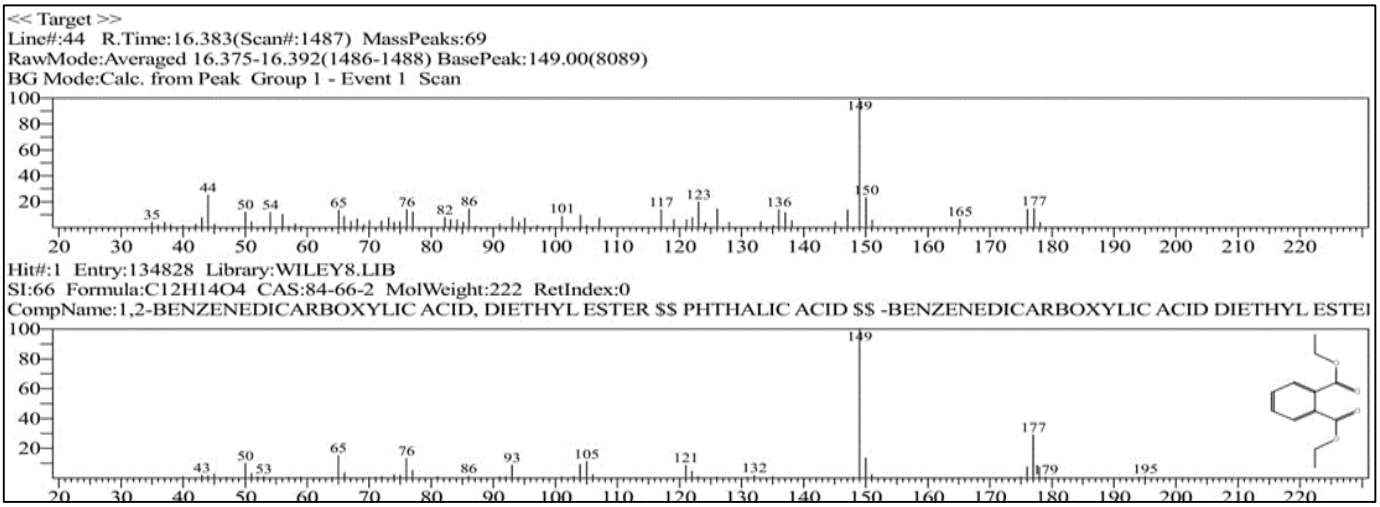
BG Mode:Calc. from Peak Group 1 - Event 1 Scan
Hit#:1 Entry:104079 Library:WILEY8.LIB
SI:91 Formula:C12H24O2 CAS:143-07-7 MolWeight:200 RetIndex:0
CompName:DODECANOIC ACID $$ AMMONIUM LAURATE $$ DODECANOATE $$ LAURIC ACID $$ LITHIUM LAURATE $$ POTASSIUM DO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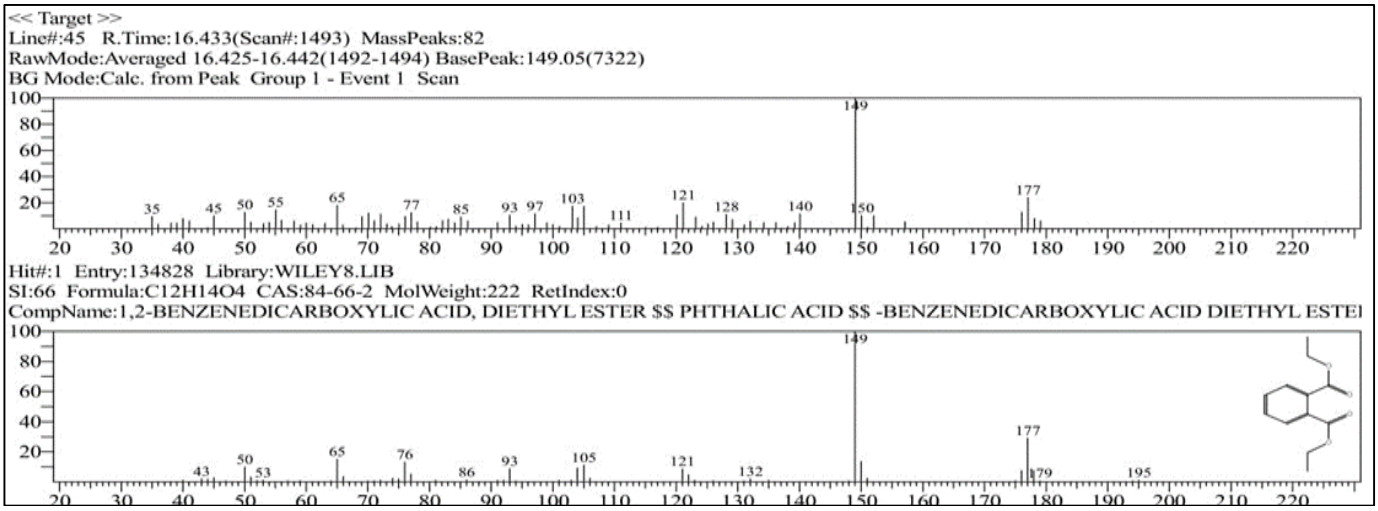
Line#:44 R.Time:16.383(Scan#:1487) MassPeaks:69
RawMode:Averaged 16.375-16.392(1486-1488) BasePeak:149.00(8089)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan
Hit#:1 Entry:134828 Library:WILEY8.LIB
SI:66 Formula:C12H14O4 CAS:84-66-2 MolWeight:222 RetIndex:0
CompName:1,2-BENZENEDICARBOXYLIC ACID, DIETHYL ESTER $$ PHTHALIC ACID $$ -BENZENEDICARBOXYLIC ACID DIETHYL ESTEI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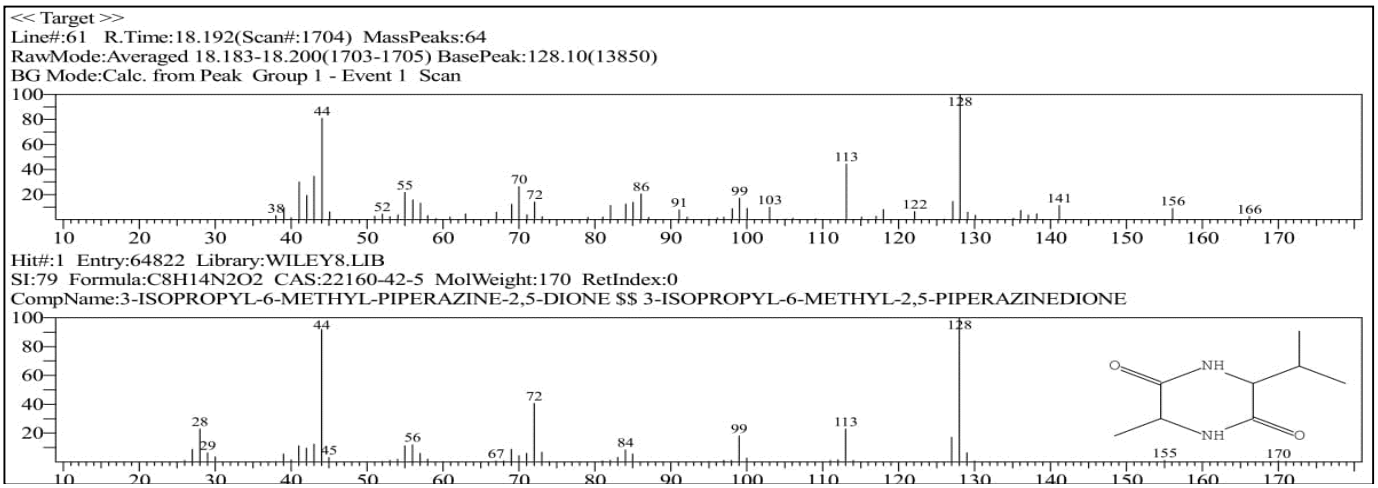
Line#:45 R.Time:16.433(Scan#:1493) MassPeaks:82
RawMode:Averaged 16.425-16.442(1492-1494) BasePeak:149.05(7322)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan
Hit#:1 Entry:134828 Library:WILEY8.LIB
SI:66 Formula:C12H14O4 CAS:84-66-2 MolWeight:222 RetIndex:0
CompName:1,2-BENZENEDICARBOXYLIC ACID, DIETHYL ESTER $$ PHTHALIC ACID $$ -BENZENEDICARBOXYLIC ACID DIETHYL ESTEI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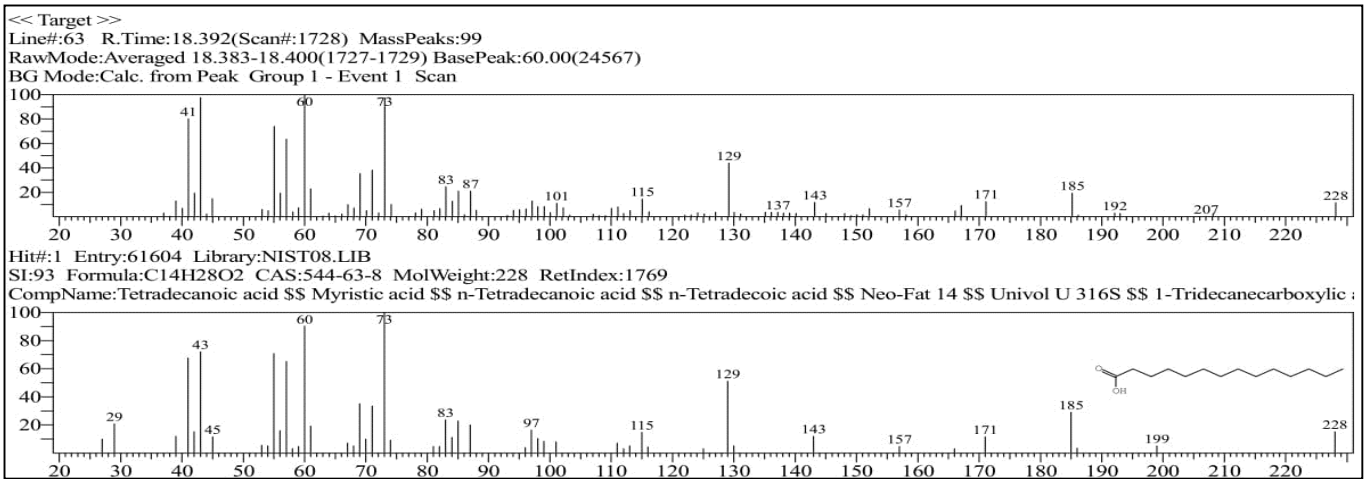
Line#:61 R.Time:18.192(Scan#:1704) MassPeaks:64
RawMode:Averaged 18.183-18.200(1703-1705) BasePeak:128.10(13850)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan
Hit#:1 Entry:64822 Library:WILEY8.LIB
SI:79 Formula:C8H14N2O2 CAS:22160-42-5 MolWeight:170 RetIndex:0
CompName:3-ISOPROPYL-6-METHYL-PIPERAZINE-2,5-DIONE $$ 3-ISOPROPYL-6-METHYL-2,5-PIPERAZINEDIONE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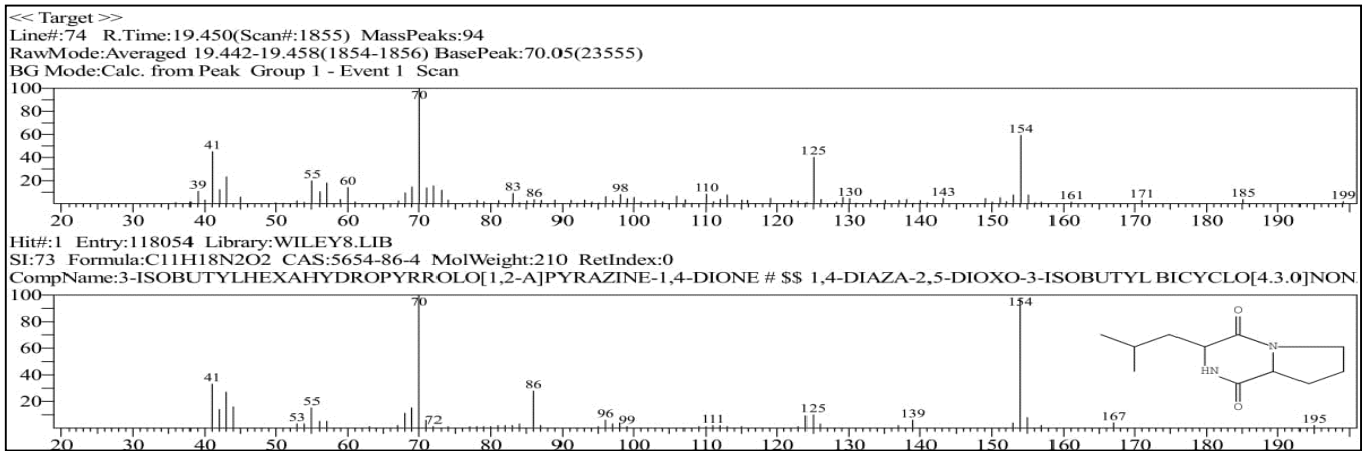
Line#:63 R.Time:18.392(Scan#:1728) MassPeaks:99
RawMode:Averaged 18.383-18.400(1727-1729) BasePeak:60.00(24567)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan
Hit#:1 Entry:61604 Library:NIST08.LIB
SI:93 Formula:C14H28O2 CAS:544-63-8 MolWeight:228 RetIndex:1769
CompName:Tetradecanoic acid $$ Myristic acid $$ n-Tetradecanoic acid $$ n-Tetradecoic acid $$ Neo-Fat 14 $$ Univol U 316S $$ 1-Tridecanecarboxylic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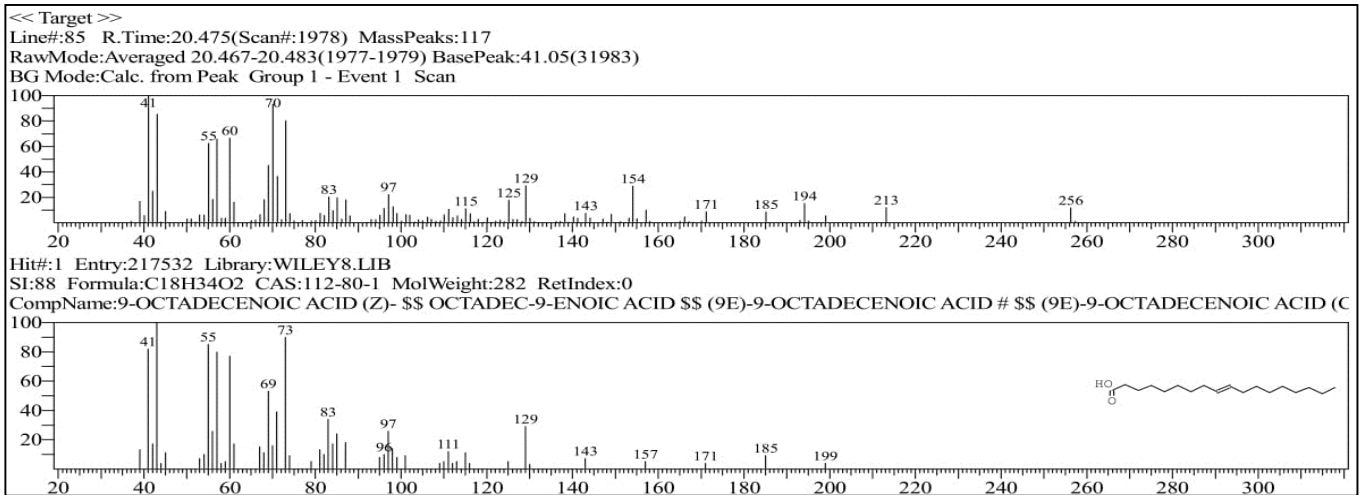
Line#:74 R.Time:19.450(Scan#:1855) MassPeaks:94
RawMode:Averaged 19.442-19.458(1854-1856) BasePeak:70.05(23555)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan
Hit#:1 Entry:118054 Library:WILEY8.LIB
SI:73 Formula:C11H18N2O2 CAS:5654-86-4 MolWeight:210 RetIndex:0
CompName:3-ISOBUTYLHEXAHYDROPYRROLO[1,2-A]PYRAZINE-1,4-DIONE # $$ 1,4-DIAZA-2,5-DIOXO-3-ISOBUTYL BICYCLO[4.3.0]NON


<< Target >>
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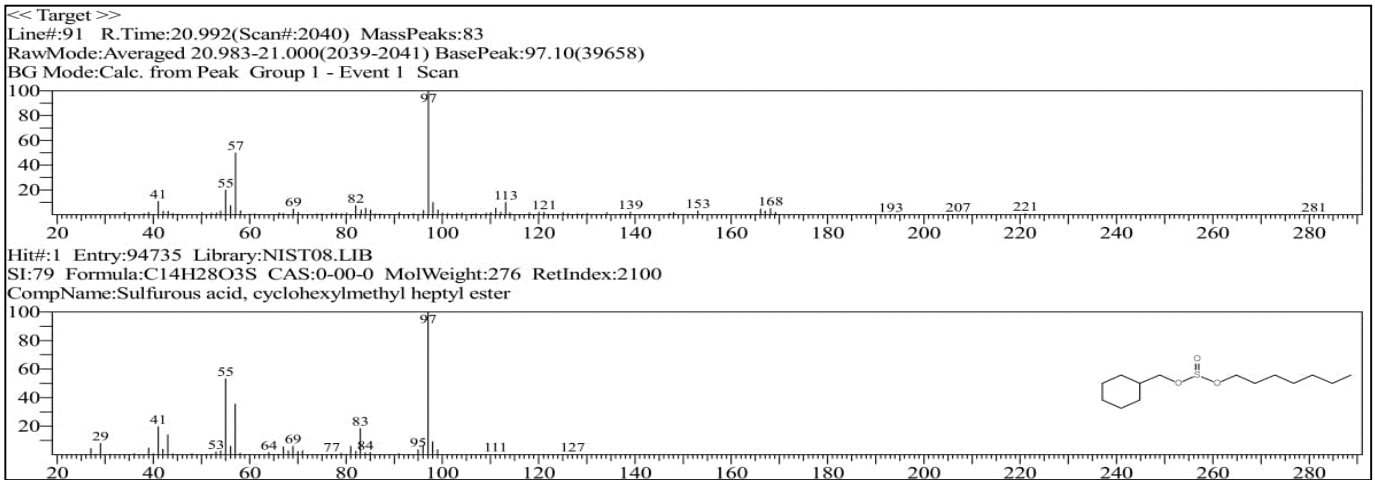
RawMode:Averaged 20.467-20.483(1977-1979) BasePeak:41.05(31983)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan
Hit#:1 Entry:217532 Library:WILEY8.LIB
SI:88 Formula:C18H34O2 CAS:112-80-1 MolWeight:282 RetIndex:0
CompName:9-OCTADECENOIC ACID (Z)- $$ OCTADEC-9-ENOIC ACID $$ (9E)-9-OCTADECENOIC ACID # $$ (9E)-9-OCTADECENOIC ACID (C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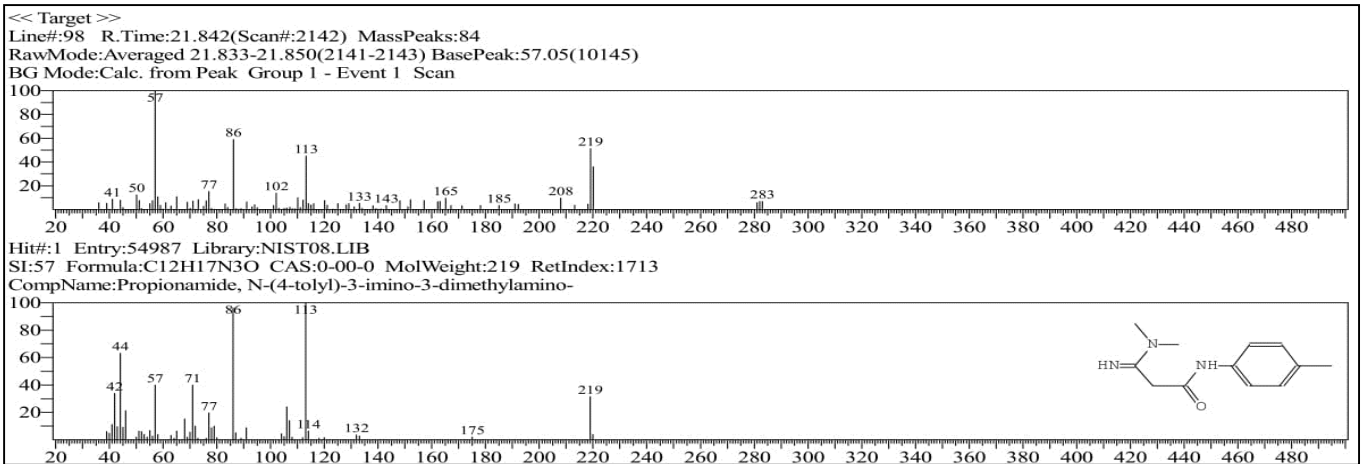
RawMode:Averaged 20.983-21.000(2039-2041) BasePeak:97.10(39658)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan
Hit#:1 Entry:94735 Library:NIST08.LIB
SI:79 Formula:C14H28O3S CAS:0-00-0 MolWeight:276 RetIndex:2100
CompName:Sulfurous acid, cyclohexylmethyl heptyl ester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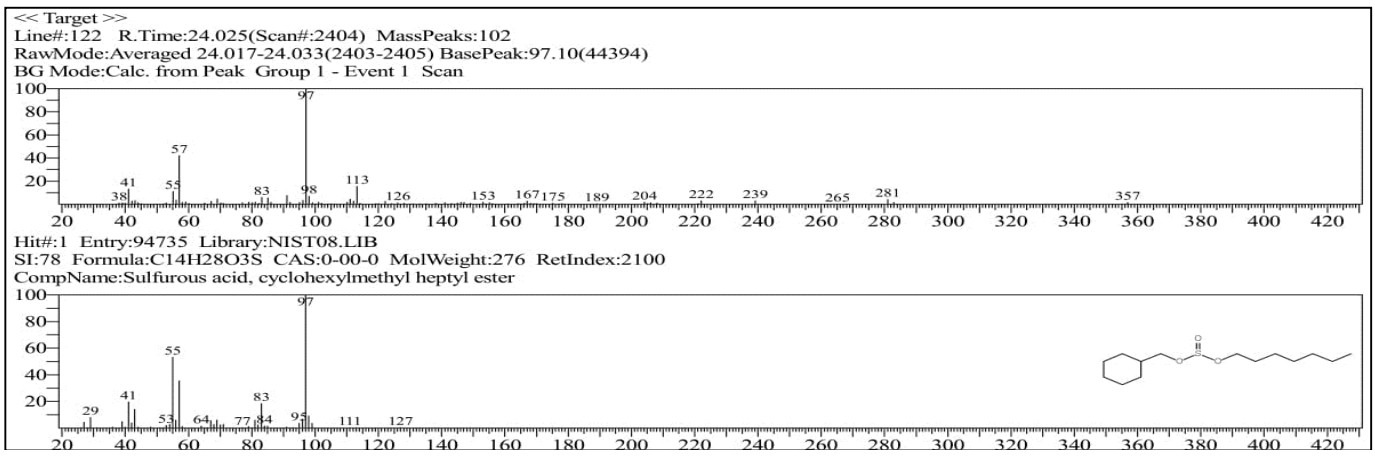
RawMode:Averaged 21.833-21.850(2141-2143) BasePeak:57.05(10145)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan
Hit#:1 Entry:54987 Library:NIST08.LIB
SI:57 Formula:C12H17N3O CAS:0-00-0 MolWeight:219 RetIndex:1713
CompName:Propionamide, N-(4-tolyl)-3-imino-3-dimethylamino-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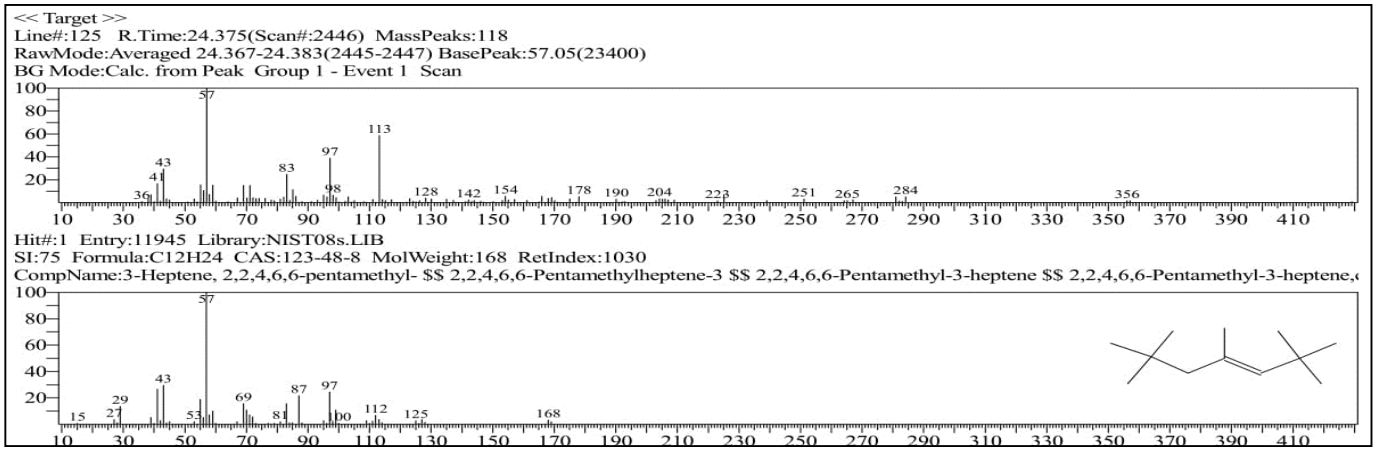
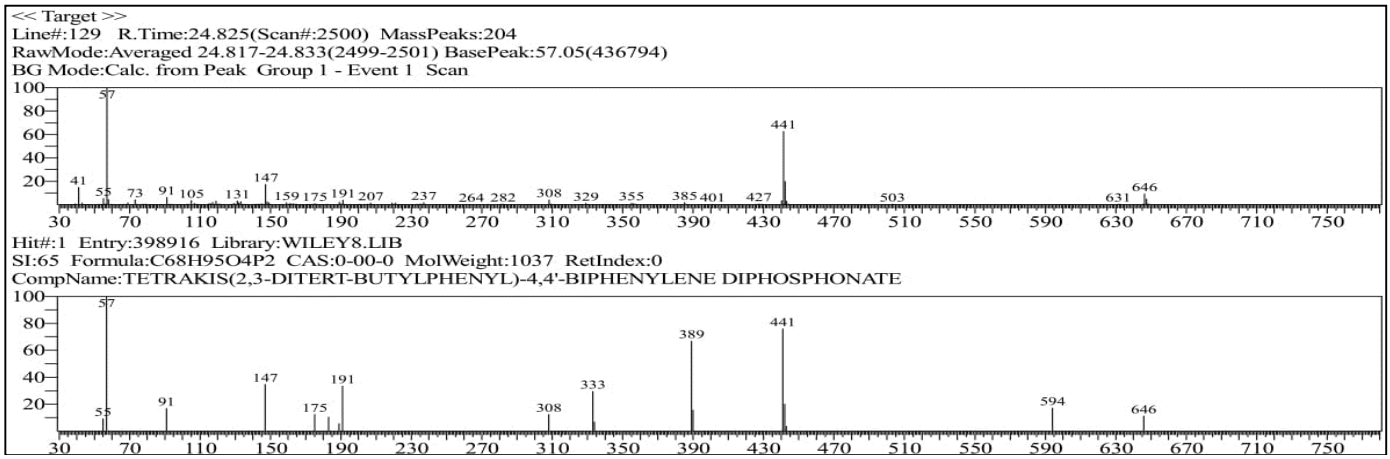
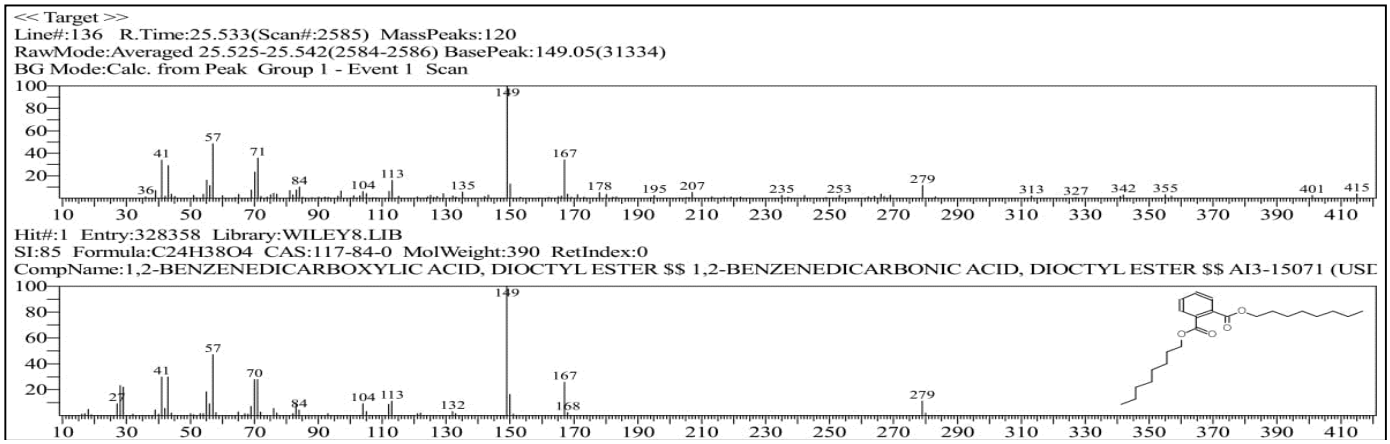
RawMode:Averaged 24.017-24.033(2403-2405) BasePeak:97.10(44394)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan
Hit#:1 Entry:94735 Library:NIST08.LIB
SI:78 Formula:C14H28O3S CAS:0-00-0 MolWeight:276 RetIndex:2100
CompName:Sulfurous acid, cyclohexylmethyl heptyl ester







Major phytochemical compounds identified in the microbial extract of *Trigonella. foenum-graecum* leaves

S. No	RT(min)	Name of the compound	Molecular Formula	Molecular Weight	Peak Area %	Pharmacological purpose
1	4.329	Silanediol, dimethyl-	$C_2H_8O_2Si$	92	2.65	No activity reported
2	6.625	Methoxy, Phenyl-Oxime-	$C_8H_9NO_2$	151	0.69	Used as Antidote for
3	8.775	Benzene acetaldehyde	C_8H_8O	120	0.45	Flavoring agent
4	16.109	Dodecanoic Acid	$C_{12}H_{24}O_2$	200	0.94	Flavoring agent, Herbicide, Insecticide, Surfactants
5	16.384 16.434	1,2-Benzenedicarboxylic acid diethyl ester	$C_{12}H_{14}O_4$	222	0.94 0.78	Flavoring agent, Phthalates, Adhesives, and sealant chemicals, Odor agents, and Plasticizers
6	18.189	3-Isopropyl-6-methyl-piperazine-2,5-dione	$C_8H_{14}N_2O_2$	170	1.16	Used as an anticonvulsant
7	18.393	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	1.89	Flavoring agent, Surfactants
8	19.450	3-Isobutyl hexahydro pyrrolo (1,2a) pyrazine	$C_{11}H_{18}N_2O_2$	210	1.28	Bitter component of sake and contributes to the flavour of bee
9	20.474	9-Octadecenoic acid (Z)	$C_{18}H_{34}O_2$	282	3.51	Flavoring agent, Food Additive
10	20.993 24.024	Sulfurous acid	$C_{14}H_{28}O_3S$	276	0.70 1.26	Bleaching Activity
11	21.839	Propionamide	$C_{12}H_{17}N_3O$	219	0.83	No activity reported
12	24.377	3-Heptene	$C_{12}H_{24}$	168	1.50	Branched unsaturated hydrocarbons
14	24.822	Tetrakis (2,3-Ditert-butylphenyl)-4,4-Biphenylene diphosphonate	$C_9H_{14}O_2$	154	24.65	stabilizer in polymers where it functions as an antioxidant
15	25.532	1,2-Benzenedicarboxylic acid	$C_{24}H_{38}O_4$	390	2.34	Plasticizers

GC-MS analysis of a microbial extract of *Trigonella* leaves detected 15 phyto compounds having antioxidant and flavoring properties. Especially Tetrakis (2, 3-Ditert-butylphenyl)-4, 4-Biphenylene diphosphonate is used as a stabilizer for polymers.

5. Conclusion

The most significant outcome of this study was the production of dark goldenrod pigment from *P. Purpurogenum* responded by producing high concentrations of the pigment of *T. Foenum* leaves. The results of the optimization, spectroscopic characterization indicates that the isolated pigment having different phytochemicals were produced having flavoring agents, antioxidant activity. The structural elucidation from GCMS shows that the 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 *Trigonella* leaves.

6. Acknowledgments

The authors are thankful to

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7. References

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