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Research Article

EXTRACTION OF PROSOPIS CINERARIA FROM BIKANER, RAJASTHAN OF INDIA-DETECTION OF COMPOUNDS VIA GCMS, CALIBRATION VIA HPLC, UV, FTIR, FESEM EDAX-AN ANTI-CANCER AGENT FOR BLOOD CANCER WITH VALIDATED IC50 VIA TESTING OVER COUNTRY EGGS

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ABSTRACT

In order to analyze the potential bioactive constituents of leaf extracts of *Prosopis Cineraria*/ *Khejri*- National tree of Rajasthan, a plant with enormous Ayurvedic properties, Fourier Transform Infrared Spectroscopy (FTIR) along with Ultra Violet Spectroscopy (UV) and added Field Emission Scanning Emission Microscope with Energy Dispersive X-ray analysis (FESEM-EDAX) were carried out. The authors too demonstrated cell line studies via MTT Assay for MOLT 4, blood cancer in different concentrations and found *Khejri* as toxic with validated IC50. Further, Gas Chromatography-Mass Spectroscopy (GC-MS) and High-Performance Liquid Chromatography (HPLC) were tested and certain compounds gave spectrum with few major peaks concluded by other results, most of which are bioactive compounds which may act as antimicrobial, anti-cancerous, antioxidant, antiviral and anti-inflammatory agents. On an all, *Khejri*/*Sami* leaves of *Mimosaceae* family of *Spunge* tree is having anti cancerous properties for blood cancer that may repair punctured blood vessels if being treated properly. The authors have demonstrated the testing over country eggs with treated/ untreated group of eggs and found the formation of blood vessels that gesture how effective the drug is, being composed of *Khejri* extract. It is not only confined to cancer diseases but acts as a very good agent for inflammation, digestion and conjunctivitis. On an all, this natural compound so extracted is found to be useful for blood cancer with IC50 as 57.11 that simply reflect that the medicine composed from the same will be highly beneficial for cancer patients - let it be Ayurvedic, homoeopathic or allopathic.

INTRODUCTION

Prosopis Cineraria *(Khejri*) is a flowering tree, found widely in the Thar Desert of Rajasthan, India of the leguminous family Fabaceae that plays a vital role in preserving the ecosystem. Not only the national tree of Rajasthan, it is the natural resource too of arid regions because of its ecological role in preventing soil erosion. In Rajasthan, it is used as a folk medicine [3,4,5] for various ailments. Locals used to boil the leaves with rice and get themselves treated if having digestion, constipation, and respiratory problems. Its bark is dry, bitter, having sharp taste, cures leprosy, dysentery, piles, asthma, leukoderma, muscle tremors and anxious mind. Its flowers are used during pregnancy as safeguard against miscarriage. Ashes of the bark are rubbed against skin to remove hairs. Smoke of leaves are good for eye problems [3,4,5]. Fresh leaves are useful for dyspepsia, crushed crude is used toothache, ear ache, fractured bones. Due to the presence of various phytoconstituents like alkaloids, steroids, phenolics, tannins and flavone derivatives, it is used as a medicine. In this regard, polyphenolic compounds have been reported to have multiple biological effects including anti-cancerous, anti-inflammatory, and anti-oxidant. GCMS is a breakthrough in the analysis of phytoconstituents and structure elucidation of the compounds as low as 1mg (Liebler et al., 1996).

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Study Area Topological Map- Bikaner, Rajasthan of India-Where sample have been collected.

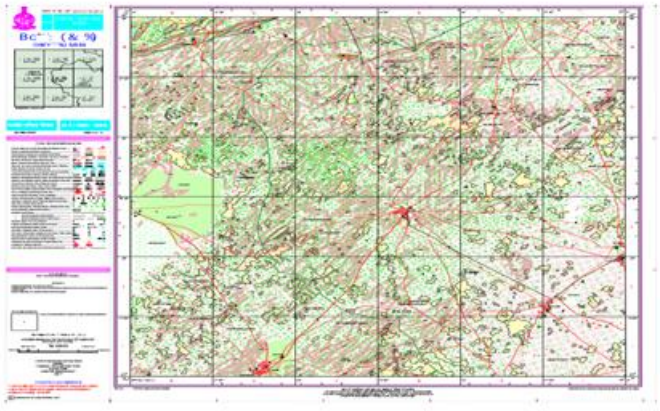
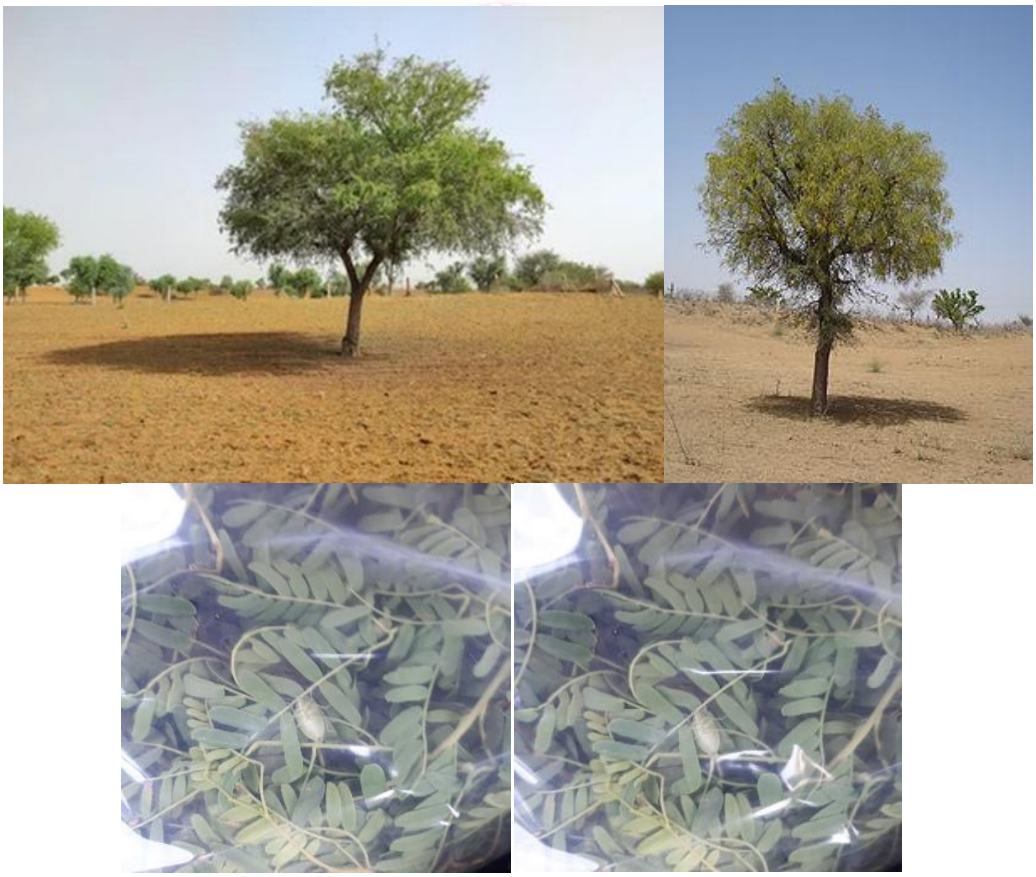


Figure 1: Elucidates the geographical toposheet of Bikaner, Rajasthan, India from samples have been collected in the month of October, 2022. Topological map assigned from Survey of India.

MATERIALS AND METHODS

Plant sample was obtained from Bikaner, Rajasthan, India, in the month of October 2022 and deposited to Lab No. 14 of Dept of Earth Sciences, Annamalai University. Binocular microscopic pictures were taken. In the process of plant extraction, material (*Khejri* leaves) was dried and powdered to 100gm, preheated sample was placed in Soxhlet apparatus for 2 to 3 days, the samples were extracted in distilled cow urine, DMSO, methanol, ethanol, chloroform, and ethyl acetate for different tests. Remains collected via extraction were sterilized and put into vials of different types for different analysis.

Morphological Images



Video URL link from where the samples have been collected-

https://drive.google.com/file/d/1vNzBTYzSHbSUTOY4VnybyLHtCjxYmeyI/view?usp=share_link

Figure 2: Prosopis Cineraria- raw leaves captured via camera, immediately after collection from Khejri tree there from Bikaner, Rajasthan, India

Binocular Microscopic Images

Figure 3: Dried leaves captured under microscope

MTT Assay for Cell Cytotoxicity

Principle- (3-4, 5 dimethylthiazol-2yl-2, 5-diphenyl tetrazolium bromide) MTT assay id the capability of mitochondrial dehydrogenase enzyme from viable cells to cleave the rings from tetrazolium pale yellow MTT and hence to form blue colored formazan crystals that are largely impermeable to cell membranes, thus outputting in its accumulation within healthy cells. By the addition of detergents like DMSO, solubilization of cells results in the liberation of crystals that are soluble. Number of surviving cells is directly proportional to the level of formazan so created. We can quantify the color using a multi-well plate reader.

Materials Required – Dulbecco's modified eagle medium (DMEM) [1,2], antibiotic solutions and Fetal Bovine serum (FBS) were brought from Gibco, USA. Dimethyl sulfoxide (DMSO) and 3-4, 5 dimethylthiazol-2yl-2, 5-diphenyl tetrazolium bromide (MTT) (5mg/ml) were landed from Sigma, USA. 1X Phosphate Buffer Saline (PBS) was from Himedia, India. Wash beakers and Tissue culture plates with 96 wells were from Tarson, India.

PROCEDURE

Cell Culture- MOLT 4 cells (Blood cancer cells) were purchased from NCCS, Pune, and were cultured in liquid medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 100µg/ml of penicillin and 100µg/ml of streptomycin and are maintained within an atmosphere of 37 degrees Celsius along with 5% of CO_2.

MTT Assay- *Khejri* leaf sample was tested for *in vitro* cytotoxicity, using MOLT 4 cells by MTT assay. Briefly, the cultured MOLT 4 cells were harvested by trypsinization, pooled in a 15ml tube. At a density of $1*10^5$ cells/ ml cells, cells were plated with cells/well (200µL) into 96- well tissue cultured plate in DMEM containing 10% of FBS and 1% of antibiotic solution for 24-48 hour at 37°C. The wells were washed with sterile PBS and treated with various concentrations of the 5 sample in a serum free DMEM medium. Each sample was in replication for three times and at 37°C, cells were incubated in a humidified 5% CO_2 for 24 hours. After incubation period, MTT (20µL of 5mg/ml) was inserted into each well and cells were incubated for another 2 to 4 hours until purple precipitates were clearly seen under inverted microscope. Finally, the medium along with MTT (220µL) were aspirated off the wells and washed after that with 1X PBS (200µl). Further-after, DMSO (100µL) was added to dissolve formazan crystals, and the plate was shaken for 4 to 5 minutes. Absorbance for each well was measured at 570nm using micro plate reader (THERMO FISHER SCIENTIFIC, USA) and IC50 and Percentage cell viability was calculated using Graph Pad Prism 6.0 Software, USA [10,11].

Formula Cell viability % = Test OD/Control OD X 100

RESULT- A

Table 1: Elucidating different concentrations with OD values at 570nm

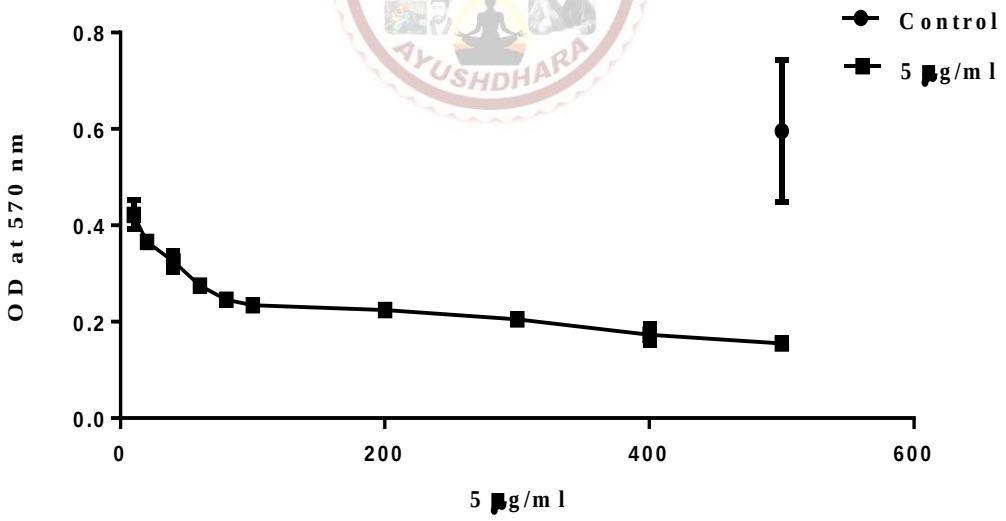
S.No	Tested sample concentration (µg/ml)	OD Value at 570nm (In triplicates)		
1.	Control	0.452	0.745	0.59
2.	500µg/ml	0.155	0.163	0.147
3.	400µg/ml	0.17	0.153	0.197
4.	300µg/ml	0.209	0.204	0.202
5.	200µg/ml	0.228	0.211	0.235

6.	100µg/ml	0.226	0.242	0.235
7.	80µg/ml	0.244	0.24	0.253
8.	60µg/ml	0.261	0.282	0.283
9.	40µg/ml	0.31	0.317	0.349
10.	20µg/ml	0.354	0.367	0.376
11.	10µg/ml	0.389	0.428	0.448

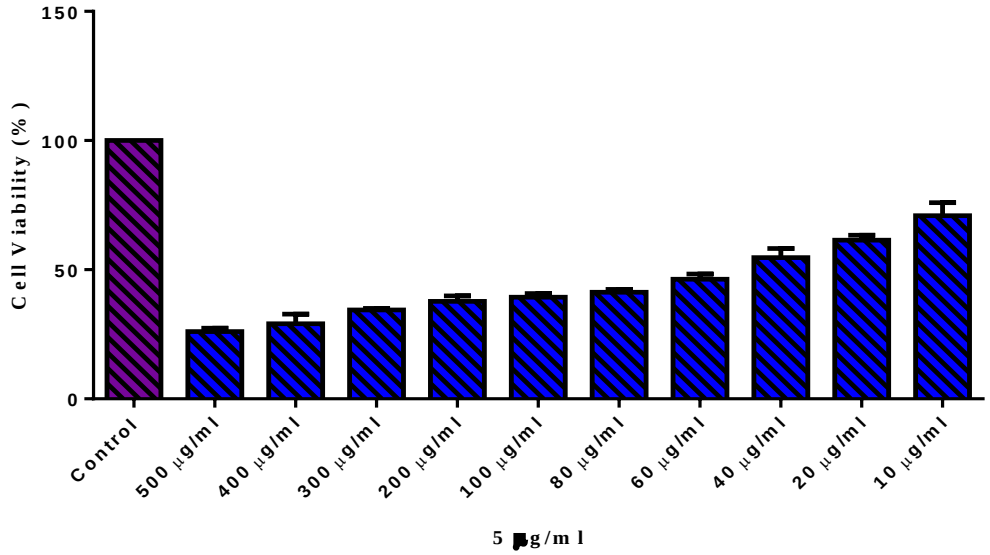
Table 2: Different concentrations with cell viability and mean

S.No	Tested sample concentration (µg/ml)	Cell viability (%) (In triplicates)	Mean Value (%)		
1.	Control	100	100	100	100
2.	500µg/ml	26.05042	27.39496	24.70588	26.05042
3.	400µg/ml	28.57143	25.71429	33.10924	29.13165
4.	300µg/ml	35.12605	34.28571	33.94958	34.45378
5.	200µg/ml	38.31933	35.46218	39.4958	37.7591
6.	100µg/ml	37.98319	40.67227	39.4958	39.38375
7.	80µg/ml	41.0084	40.33613	42.52101	41.28852
8.	60µg/ml	43.86555	47.39496	47.56303	46.27451
9.	40µg/ml	52.10084	53.27731	58.65546	54.67787
10.	20µg/ml	59.4958	61.68067	63.19328	61.45658
11.	10µg/ml	65.37815	71.93277	75.29412	70.86835

Cell viability (%)



Plot 1: OD at 570nm with 5 µg/ml concentrations



Plot 2: Cell viability at different concentrations

IC50 Value of tested sample: 57.72µg/ml

Table 3: Statistical outputs via Elisa, Graph Prism with IC50

log(inhibitor) vs. normalized response Variable-slope		
Best-fit values		
LogIC50		1.761
Hill-Slope		-1.305
IC50		57.72
Std. Error		
LogIC50		0.02820
Hill Slope		0.1135
95% Confidence Intervals		
LogIC50		1.704 to 1.819
Hill-Slope		-1.537 to -1.072
IC50		50.53 to 65.93
Goodness of Fit		
Degrees of Freedom		28
R square		0.9400
Absolute Sum of Squares		1698
Sy.x		7.786
Number of points		
Analyzed	3	30

Images of control cells and 5 treated cells

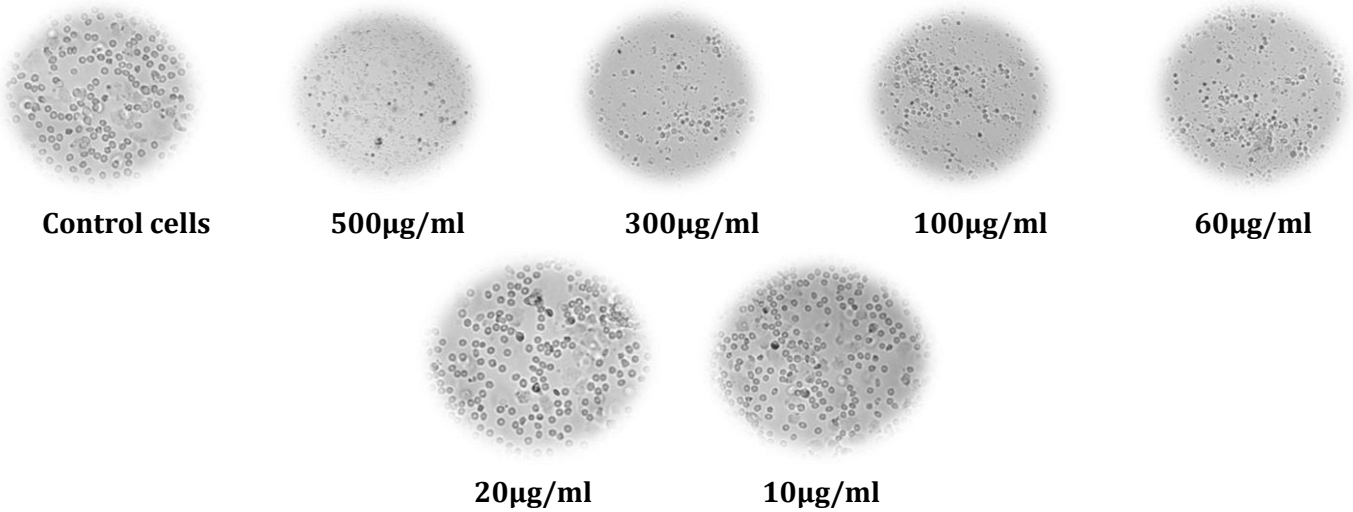
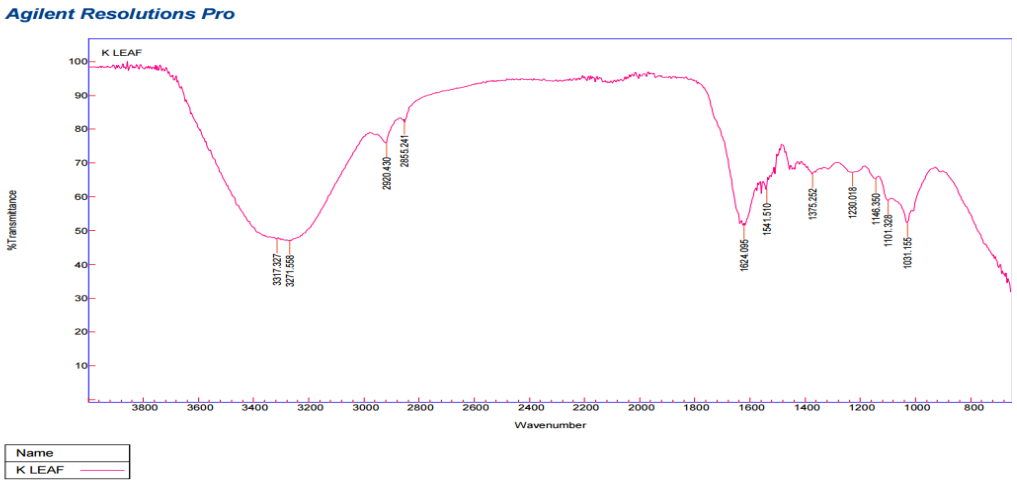


Figure 4: Morphological cancer cells MOLT 4 images at Untreated and Treated levels

Fourier Transform Infrared Spectroscopy (FTIR)- These spectrometers are just like NDIR analyzers, i.e., the fact that many gases absorb IR radiation at species- specific frequencies. It is a disperse method that gestures the measurements are performed over a broad spectrum instead of a narrow band of frequencies. This spectroscopy takes advantage of how IR light changes the dipole moments in molecules that corresponds to a specific vibration energy.



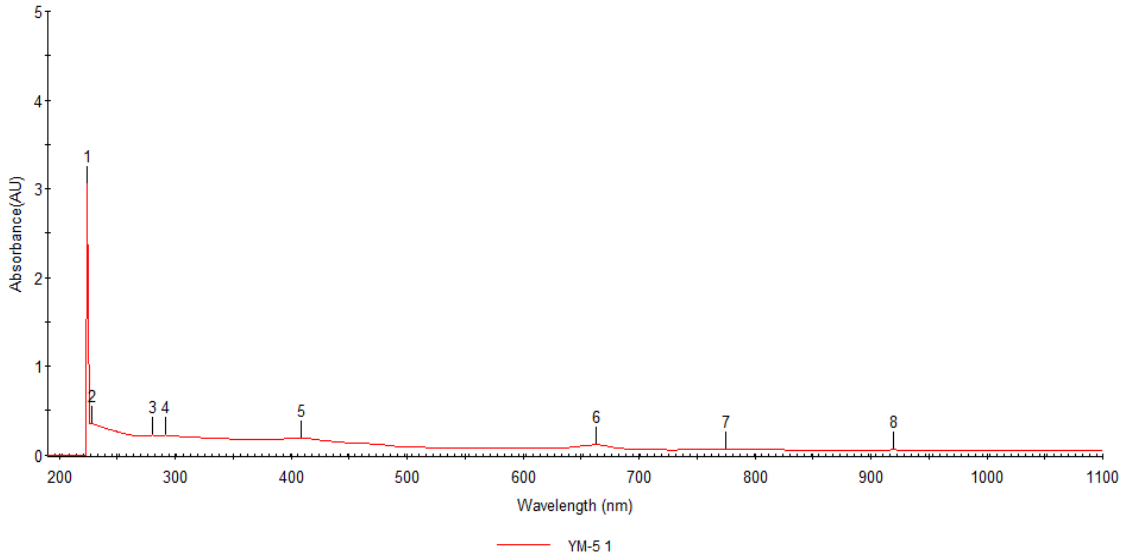
Plot 3: Infrared peaks samples using FTIR Spectroscopy

IR Spectrum Table

Table 4: Conclusion table from Infrared Spectrum

Wave Number (Highest Peaks)	Transmittance % Age	Functional Class	Stretching Vibration
3317	49	Alcohols/Phenols	O-H (free), usually sharp
3211	49	Alcohols/Phenols	O-H (H-bonded), usually broad
2920	78	Alkanes	C-H stretch

UV Spectroscopy- Ultra Violet Spectroscopy is used in bio or analytical chemistry for the quantitative determination of analytes, such as transition metal ions, biological macromolecules, and highly conjugated organic compounds. This technique is based on the principle of electronic transition in atoms or molecules that is caused by absorption of light in the visible area of electromagnetic spectrum (400-800nm) under excitation of an electron from the ground state into a higher orbital. UV region covers the wavelength range 100-400nm and is divided into 3 bands: UVA (315-400nm), UVB (280-315nm) and UVC (100-280nm).



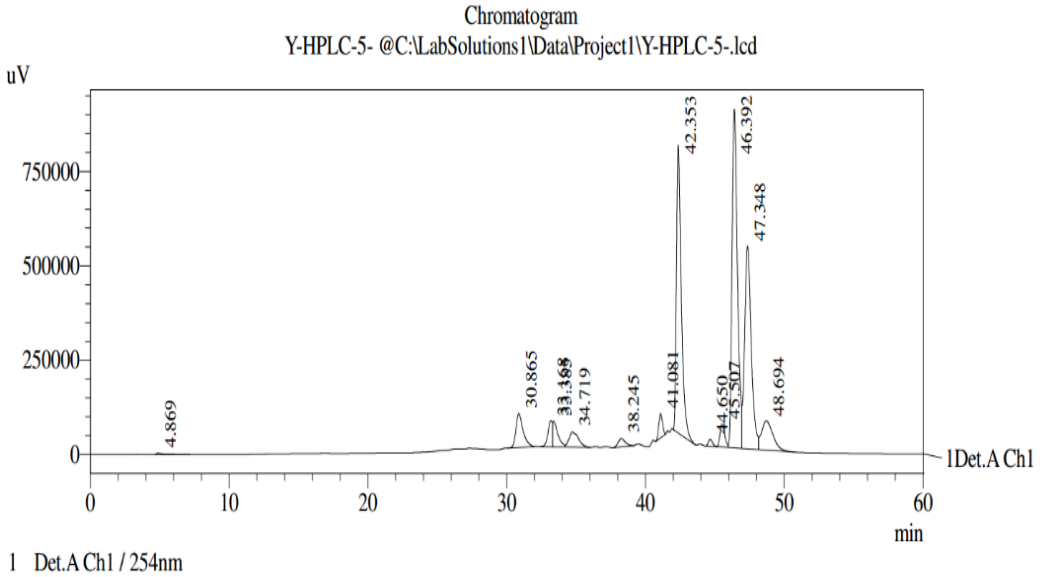
Plot 4: Ultraviolet Spectrum peaks at different wavelengths

Table 5: Different peaks in nanometer with AU

No.	1	2	3	4	5	6	7	8
Peak(nm)	224.25	227.95	279.90	292.25	408.75	663.25	775.35	918.55
Peak (AU)	3.789	0.353	0.222	0.222	0.186	0.114	0.062	0.056

High Performance Liquid Chromatography (HPLC)- High Performance Liquid Chromatography or High-Pressure Liquid chromatography is a chromatographic method that is used to separate a series of compounds in bio and analytical chemistry in order to purify, identify and quantify the individual components of the mixture. This detection is primarily based on the nondestructive detection such as UV, photodiode array detectors, RI, Conductivity, and laser detection. On the contrary, gas chromatography detection [17] is based highly on destructive principles like NPD, FID and FPD.

Chromatogram [8,9]



PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.869	98746	3777	0.127	0.139
2	30.865	3236139	89772	4.176	3.310
3	33.168	1502632	69160	1.939	2.550
4	33.385	1770972	67650	2.286	2.494
5	34.719	1928503	41163	2.489	1.518
6	38.245	800029	22291	1.032	0.822
7	41.081	1194625	64436	1.542	2.376
8	42.353	18482650	762725	23.853	28.123
9	44.650	379820	18382	0.490	0.678
10	45.507	1418489	60440	1.831	2.228
11	46.392	24330805	897562	31.401	33.094
12	47.348	17911048	537184	23.116	19.807
13	48.694	4430279	77579	5.718	2.860
Total		77484736	2712119	100.000	100.000

Figure 5: High Performance Liquid Chromatography peaks with Area and Height

Field Emission Scanning Electron Microscope Energy Dispersive X-ray Spectroscopy (FESEM EDAX)- The primary principle of EDAX is a generation of X-rays from a specimen through the electron beam. This technique can also be used measure the energy of emitted rays. FESEM provides probing beams at high as well as low electron energy. FESEM EDX investigations enable us to reveal the correlation between morphological changes and elemental composition. Aging processes were clarified by learning changes in the elemental composition to the fiber core in cross-sections.

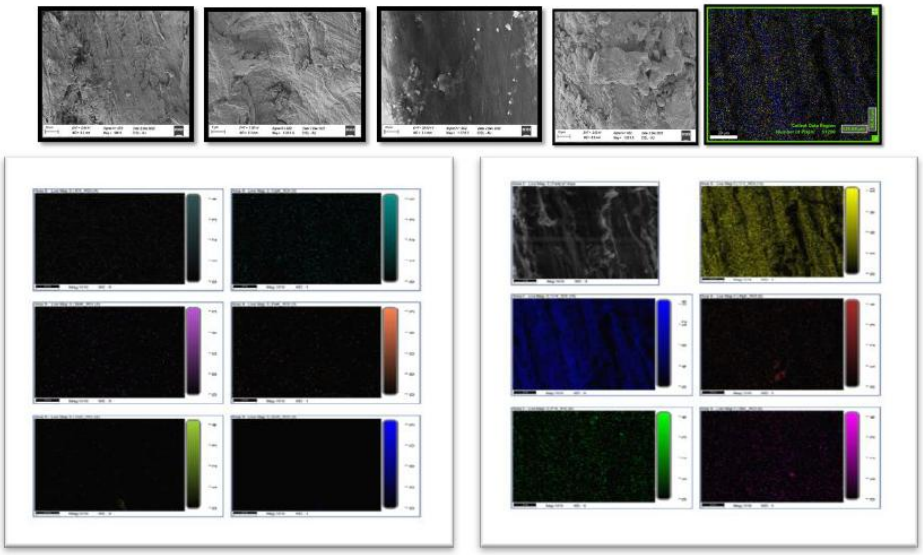
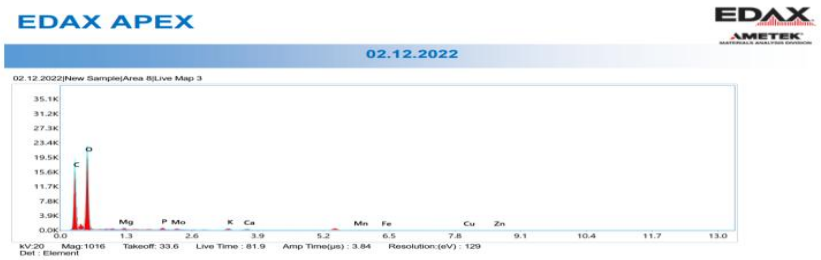


Figure 6: FESEM with Mapping of different elements



eZAF Quant Result - Analysis Uncertainty: 14.16 %

Element	Weight %	MDL	Atomic %	Error %	Net Int.	R	A	F
C K	51.86	0.1	59.40	9.77	1170.26	0.9341	0.1472	1.0000
O K	46.61	0.06	40.07	10.30	1650.60	0.9422	0.0905	1.0000
Mg K	0.30	0.02	0.17	9.73	58.60	0.9540	0.3979	1.0021
P K	0.23	0.01	0.10	5.96	59.10	0.9612	0.7670	1.0076
K K	0.28	0.02	0.10	5.98	53.10	0.9692	0.9398	1.0260
Ca K	0.20	0.02	0.07	7.52	32.65	0.9710	0.9555	1.0315
Mn K	0.09	0.03	0.02	16.92	8.85	0.9792	0.9899	1.1171
Fe K	0.01	0.03	0.00	62.91	1.06	0.9808	0.9923	1.1420
Cu K	0.04	0.04	0.01	60.00	2.48	0.9852	0.9954	1.2568
Zn K	0.03	0.05	0.01	60.59	1.46	0.9867	0.9971	1.3049
Mo L	0.34	0.03	0.05	7.89	35.68	0.9640	0.8364	1.0029

Figure 7: EDAX results of elemental composition

GCMS-Gas Chromatography Mass Spectroscopy: This test breaks mass spectroscopy into each separated compound that came from gas chromatography into ionized fragments [12,13]. To perform this, a high energy beam of electrons is passed through the sample molecule to produce electrically charged ions or particles. These fragments can be small or large pieces of the original molecule. The heated gases are carried through the column with inert gases, as for e.g. helium.

The identification of compounds was done using computer matching of mass spectra [6,7] with those of standards (WILEY8. LIB. and NIST11.LIB). Samples were analyzed in GCMS-QP2020 Plus from Bishop Heber College, Trichy, India.

Table 6: Detailing of GCMS in Elaboration

Method

[Comment]

===== Analytical Line 1 =====

[AOC-20i]

# of Rinses with Presolvent	:3
# of Rinses with Solvent(post)	:3
# of Rinses with Sample	:3
Plunger Speed(Suction)	:High
Viscosity Comp. Time	:0.2 sec
Plunger Speed(Injection)	:High
Syringe Insertion Speed	:High
Injection Mode	:Normal
Pumping Times	:5
Inj. Port Dwell Time	:0.3 sec
Terminal Air Gap	:No
Plunger Washing Speed	:High
Washing Volume	:6uL
Syringe Suction Position	:0.0 mm
Syringe Injection Position	:0.0 mm
Use 3 Solvent Vial	:1 vial

[GC-2010]

Column Oven Temp.	:50.0 °C
Injection Temp.	:250.00 °C
Injection Mode	:Split
Flow Control Mode	:Linear Velocity
Pressure	:68.1 kPa
Total Flow	:16.2 mL/min
Column Flow	:1.20 mL/min
Linear Velocity	:39.7 cm/sec
Purge Flow	:3.0 mL/min
Split Ratio	:10.0
High Pressure Injection	:OFF
Carrier Gas Saver	:OFF
Splitter Hold	:OFF

Oven Temp. Program

Rate	Temperature(°C)	Hold Time(min)
-	50.0	0.00
6.00	280.0	2.00

< Ready Check Heat Unit >

Column Oven	: Yes
SPL1	: Yes
MS	: Yes

< Ready Check Detector(FTD/BID) >
< Ready Check Baseline Drift >
< Ready Check Injection Flow >

SPL1 Carrier	: Yes
SPL1 Purge	: Yes

< Ready Check APC Flow >
< Ready Check Detector APC Flow >

External Wait	:No
Equilibrium Time	:0.5 min

[GC Program]

[GCMS-QP2020]

IonSourceTemp	:200.00 °C
Interface Temp.	:250.00 °C
Solvent Cut Time	:3.50 min
Detector Gain Mode	:Relative to the Tuning Result
Detector Gain	:+0.00 kV
Threshold	:1000

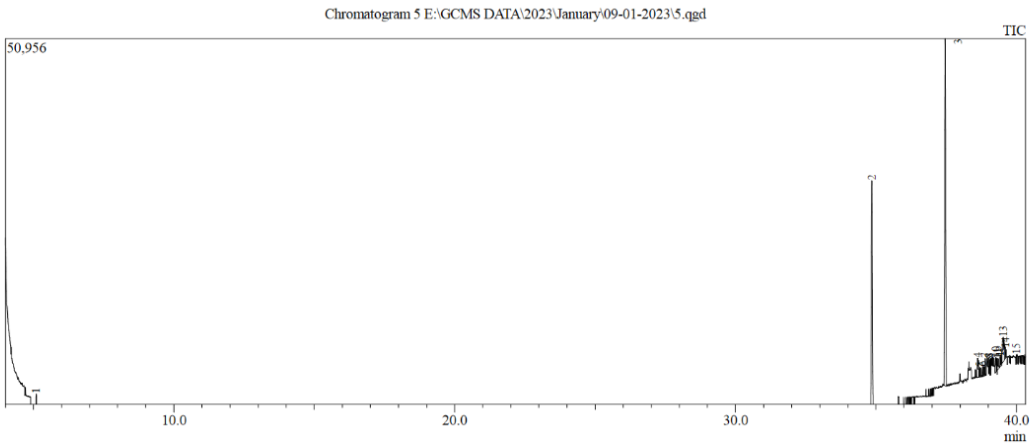
[MS Table]
--Group 1 - Event 1--

Start Time	:4.00min
End Time	:40.33min
ACQ Mode	:Scan
Event Time	:0.30sec
Scan Speed	:1666
Start m/z	:50.00
End m/z	:500.00
Sample Inlet Unit	:GC

[MS Program]
Use MS Program :OFF

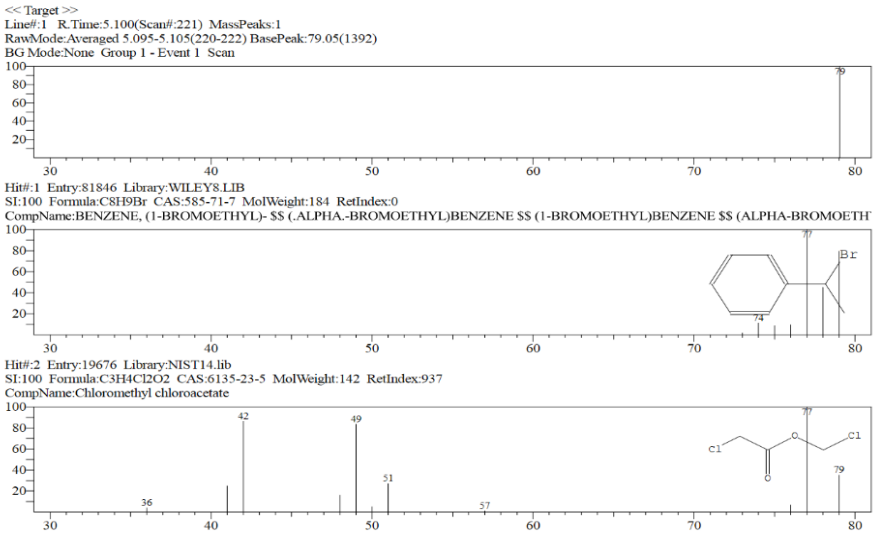
Peak	Retention	Start time	End Time	m/z	Area	Area %	Height	Height %	A/H	Mark	Name
1	5.1	5.085	5.115	TIC	1901	0.85	1463	1.41	1.3		BENZENE, (1-BROMOETHYL)-
2	34.851	34.815	34.9	TIC	64770	29.09	31210	30.03	2.08		1,2-BENZENEDICARBOXYLIC ACID, DIISOOCTYL ESTER
3	37.466	37.42	37.51	TIC	103198	46.36	48329	46.5	2.14		1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester
4	38.622	38.585	38.665	TIC	7050	3.17	2686	2.58	2.62		(E,E)-2-[3-(DIMETHYLAMINO)-2-PROPYLIDENE]CYCLOHEPTANONE
5	38.7	38.665	38.78	TIC	3007	1.35	1289	1.24	2.33	V	D-GLUCO-HEPTONIC ACID, 2,6-ANHYDRO-3-DEOXY-, METHYL ESTER
6	38.797	38.78	38.81	TIC	1768	0.79	1346	1.3	1.31	V	CESIUM TRIMETHYLFLUORO)ALUMINATE
7	38.893	38.81	38.915	TIC	4775	2.14	2386	2.3	2		BENZENEBUTANETHIOIC ACID, .ALPHA.,.ALPHA.,2,4,6-PENTAMETHYL-, S-ETHYL ESTER
8	39.023	39.01	39.03	TIC	1855	0.83	2128	2.05	0.87		(S)-1,1-DIMETHYLETHYL 3-HYDROXY-2-METHYL-5-PHENYL-4-PENTENEDITHIOATE
9	39.055	39.03	39.065	TIC	2555	1.15	2122	2.04	1.2	V	TRANS-9-FLUORO-2,2,3,3-TETRAMETHOXY-7,7-DIMETHYL-1-OXASPIRO[3,5]NON-5-ENE
10	39.267	39.245	39.295	TIC	3939	1.77	2012	1.94	1.96		BENZENEBUTANETHIOIC ACID, .ALPHA.,.ALPHA.,2,4,6-PENTAMETHYL-, S-ETHYL ESTER
11	39.325	39.295	39.405	TIC	4213	1.89	1524	1.47	2.76	V	1-CYCLOHEXYL-5-METHYL-3-(P-TOLYLCARBONYL)PYRROLE
12	39.415	39.405	39.49	TIC	5150	2.31	1389	1.34	3.71		(+-)-1-(ACETOXY)-2-(1-BROMOETHYL)-3-METHOXYANTHRAQUINONE
13	39.53	39.49	39.58	TIC	13955	6.27	3393	3.26	4.11	V	CESIUM TRIMETHYLFLUORO)ALUMINATE
14	39.61	39.58	39.63	TIC	3423	1.54	1394	1.34	2.46	V	(S)-1,1-DIMETHYLETHYL 3-HYDROXY-2-METHYL-5-PHENYL-4-PENTENEDITHIOATE
15	40	39.985	40.01	TIC	1066	0.48	1255	1.21	0.85		(+-)-1-(ACETOXY)-2-(1-BROMOETHYL)-3-METHOXYANTHRAQUINONE

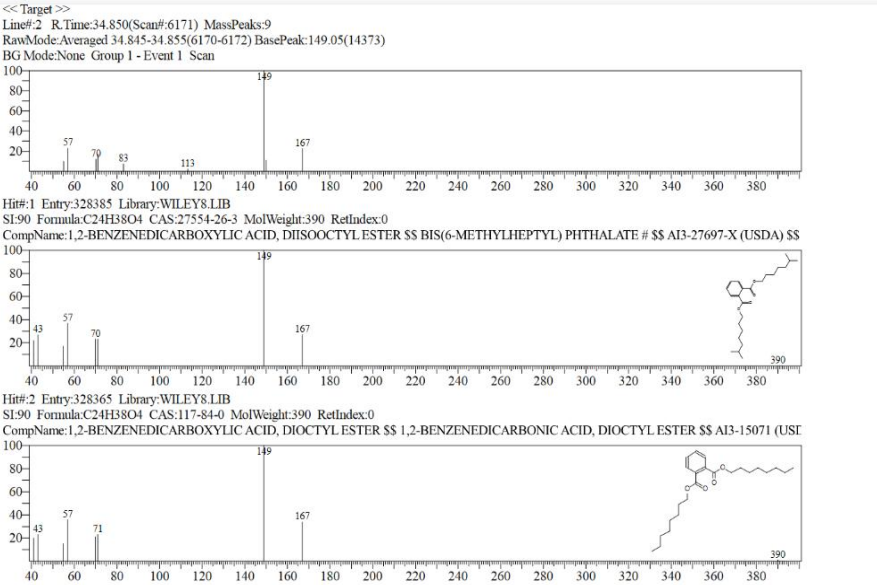
Figure 8: Compounds Identified by GCMS Analysis

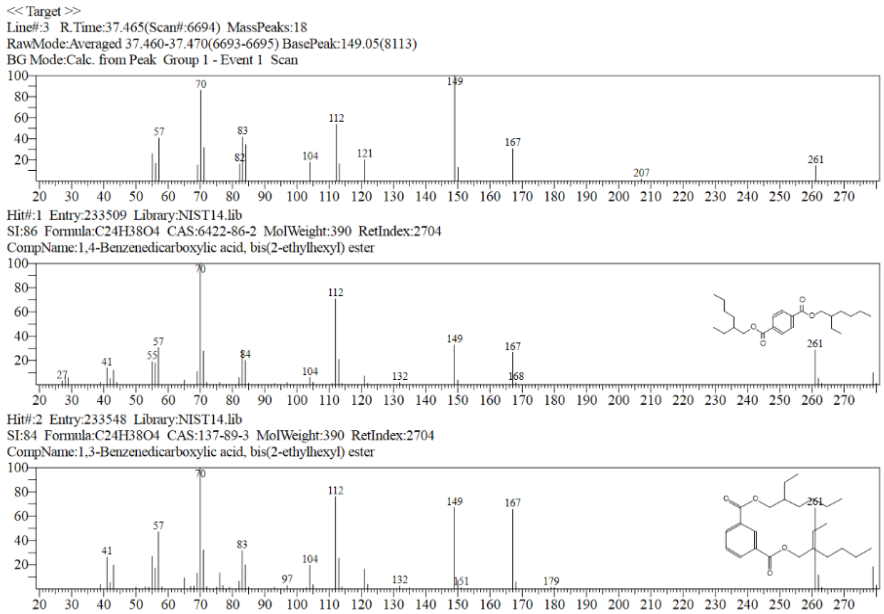


Plot 6: Peaks of Chromatogram via GCMS.

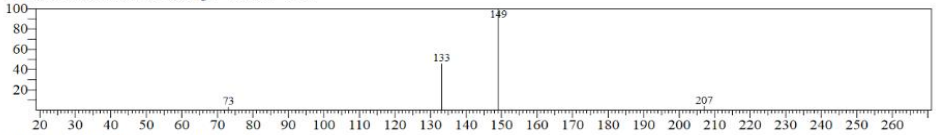
Library Functions



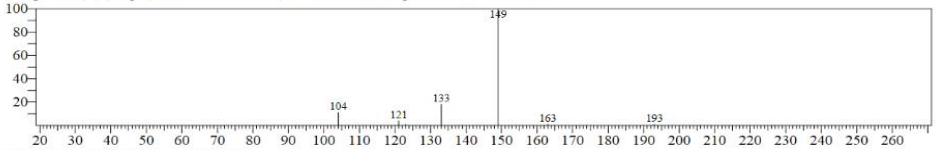




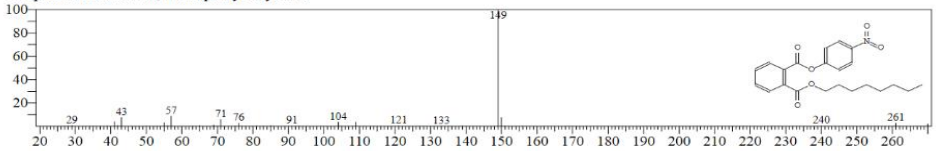
<< Target >>
Line#:4 R.Time:38.620(Scan#:6925) MassPeaks:4
RawMode:Averaged 38.615-38.625(6924-6926) BasePeak:149.05(1477)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan



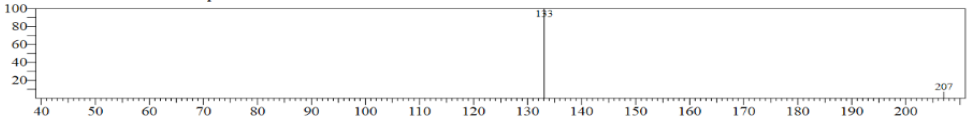
Hit#:1 Entry:93909 Library:WILEY8.LIB
SI:86 Formula:C12H19NO CAS:78804-85-0 MolWeight:193 RetIndex:0
CompName:(E,E)-2-[3-(DIMETHYLAMINO)-2-PROPYLIDENE]CYCLOHEPTANONE



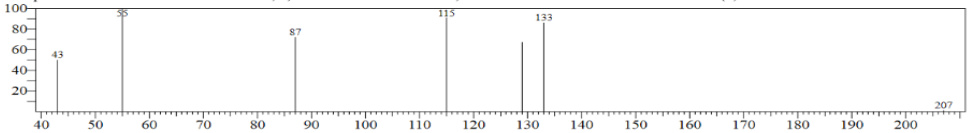
Hit#:2 Entry:238139 Library:NIST14.lib
SI:79 Formula:C22H25NO6 CAS:0-00-0 MolWeight:399 RetIndex:3105
CompName:Phthalic acid, 4-nitrophenyl octyl ester



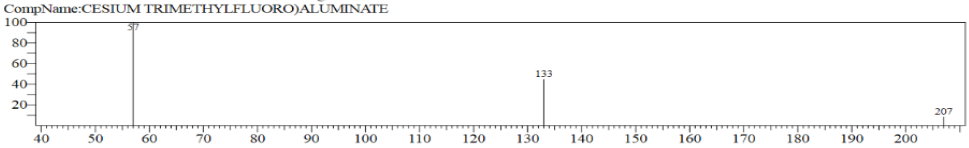
<< Target >>
Line#:5 R.Time:38.700(Scan#:6941) MassPeaks:2
RawMode:Averaged 38.695-38.705(6940-6942) BasePeak:133.05(1059)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan



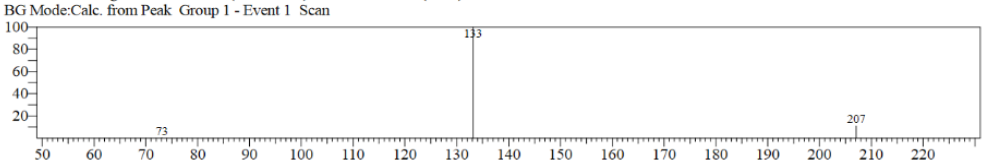
Hit#:1 Entry:111480 Library:WILEY8.LIB
SI:99 Formula:C8H14O6 CAS:108890-37-5 MolWeight:206 RetIndex:0
CompName:D-GLUCO-HEPTONIC ACID, 2,6-ANHYDRO-3-DEOXY-, METHYL ESTER $$ METHYL .BETA.-(2,6-ANHYDRO-3-DEOXY-D-ARABI



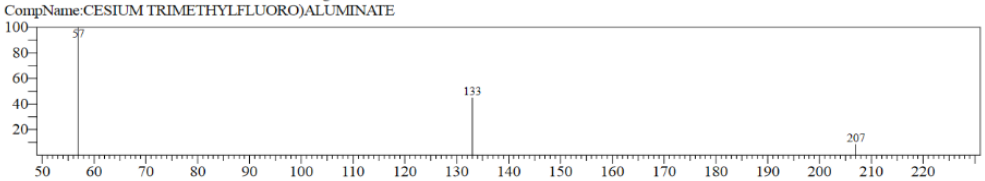
Hit#:2 Entry:137461 Library:WILEY8.LIB
SI:97 Formula:C3H9AlCsF CAS:0-00-0 MolWeight:224 RetIndex:0
CompName:CESIUM TRIMETHYLFLUORO)ALUMINATE



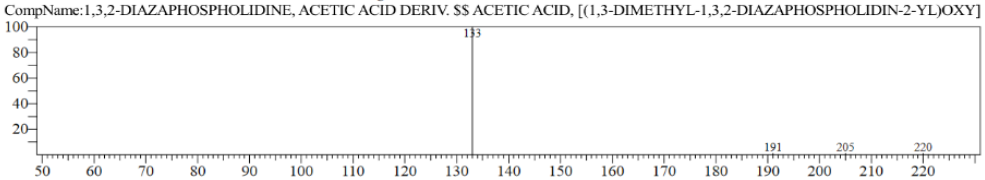
<< Target >>
Line#:6 R.Time:38.795(Scan#:6960) MassPeaks:3
RawMode:Averaged 38.790-38.800(6959-6961) BasePeak:133.10(1036)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan



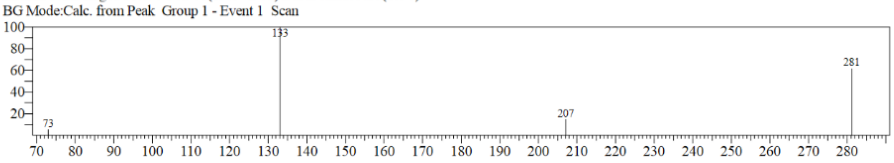
Hit#:1 Entry:137461 Library:WILEY8.LIB
SI:98 Formula:C3H9AlCsF CAS:0-00-0 MolWeight:224 RetIndex:0
CompName:CESIUM TRIMETHYLFLUORO)ALUMINATE



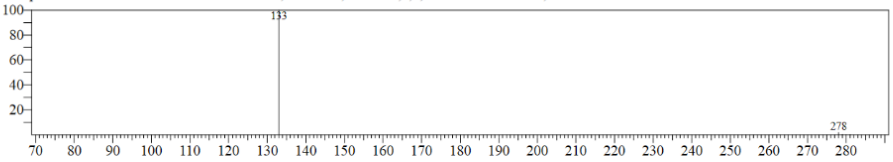
Hit#:2 Entry:131150 Library:WILEY8.LIB
SI:96 Formula:C8H17N2O3P CAS:117357-61-6 MolWeight:220 RetIndex:0
CompName:1,3,2-DIAZAPHOSPHOLIDINE, ACETIC ACID DERIV. $$ ACETIC ACID, [(1,3-DIMETHYL-1,3,2-DIAZAPHOSPHOLIDIN-2-YL)OXY]



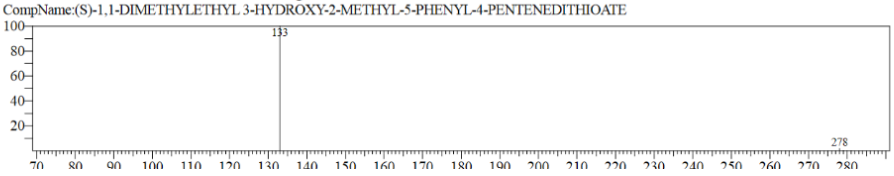
<< Target >>
Line#:7 R.Time:38.895(Scan#:6980) MassPeaks:4
RawMode:Averaged 38.890-38.900(6979-6981) BasePeak:133.10(1098)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan



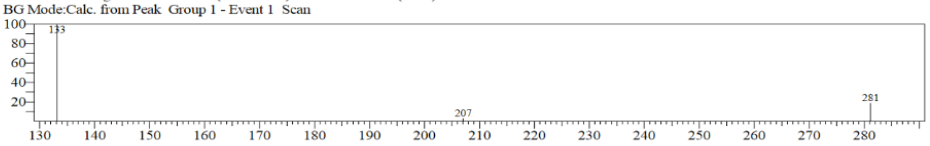
Hit#:1 Entry:212030 Library:WILEY8.LIB
SI:81 Formula:C17H26OS CAS:112303-78-3 MolWeight:278 RetIndex:0
CompName:BENZENEBUTANETHIOIC ACID, .ALPHA.,.ALPHA.,2,4,6-PENTAMETHYL-, S-ETHYL ESTER



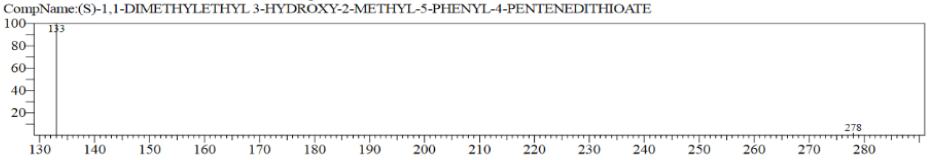
Hit#:2 Entry:211731 Library:WILEY8.LIB
SI:81 Formula:C16H22O2S CAS:0-00-0 MolWeight:278 RetIndex:0
CompName:(S)-1,1-DIMETHYLETHYL 3-HYDROXY-2-METHYL-5-PHENYL-4-PENTENEDITHIOATE



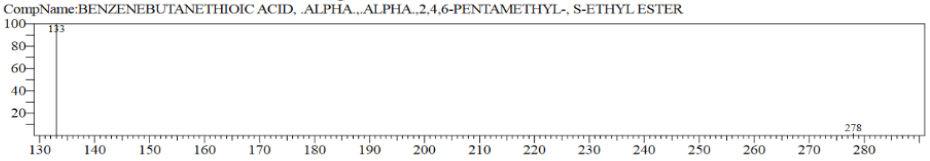
<< Target >>
Line#:8 R.Time:39.020(Scan#:7005) MassPeaks:3
RawMode:Averaged 39.015-39.025(7004-7006) BasePeak:133.10(1048)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan



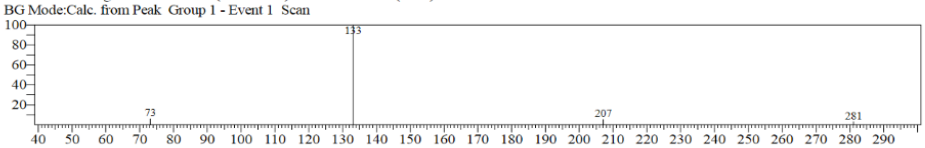
Hit#:1 Entry:211731 Library:WILEY8.LIB
SI:94 Formula:C16H22O2S CAS:0-00-0 MolWeight:278 RetIndex:0
CompName:(S)-1,1-DIMETHYLETHYL 3-HYDROXY-2-METHYL-5-PHENYL-4-PENTENEDITHIOATE



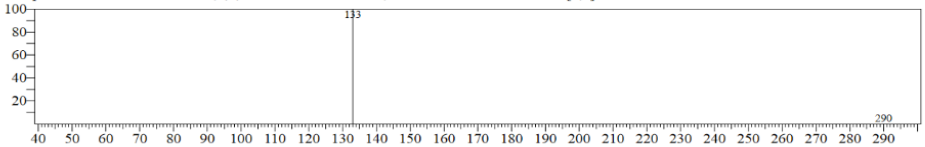
Hit#:2 Entry:212030 Library:WILEY8.LIB
SI:94 Formula:C17H26OS CAS:112303-78-3 MolWeight:278 RetIndex:0
CompName:BENZENEBUTANETHIOIC ACID, .ALPHA.,.ALPHA.,2,4,6-PENTAMETHYL-, S-ETHYL ESTER



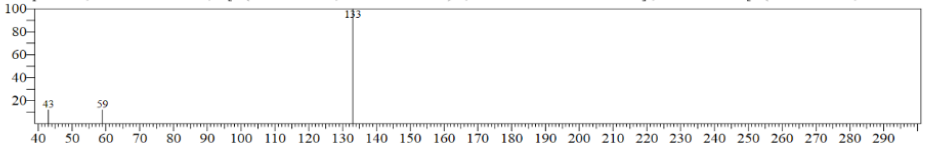
Line#:9 R.Time:39.055(Scan#:7012) MassPeaks:4
RawMode:Averaged 39.050-39.060(7011-7013) BasePeak:133.05(1029)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan



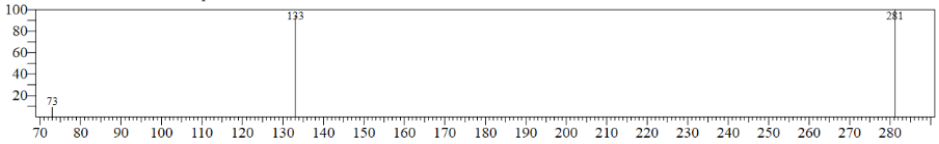
Hit#:1 Entry:226959 Library:WILEY8.LIB
SI:93 Formula:C14H23FO5 CAS:0-00-0 MolWeight:290 RetIndex:0
CompName:TRANS-9-FLUORO-2,2,3,3-TETRAMETHOXY-7,7-DIMETHYL-1-OXASPIRO[3,5]NON-5-ENE



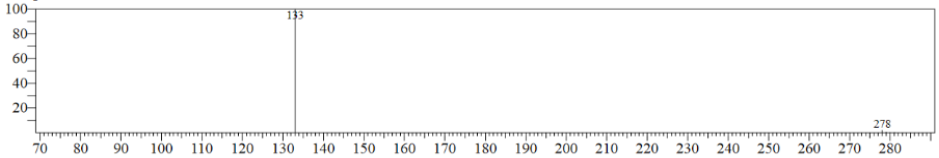
Hit#:2 Entry:267852 Library:WILEY8.LIB
SI:93 Formula:C17H24O2S2 CAS:129296-24-8 MolWeight:324 RetIndex:0
CompName:1,5-HEXANEDIONE, 1-[6-(2-METHYL-1,3-DITHIAN-2-YL)-2,4-CYCLOHEXADIEN-1-YL]-, TRANS- $$ 1-[6-(2-METHYL-1,3-DITHIA



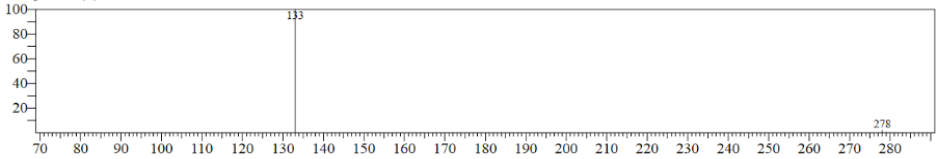
<< Target >>
Line#:10 R.Time:39.265(Scan#:7054) MassPeaks:3
RawMode:Averaged 39.260-39.270(7053-7055) BasePeak:281.15(739)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan



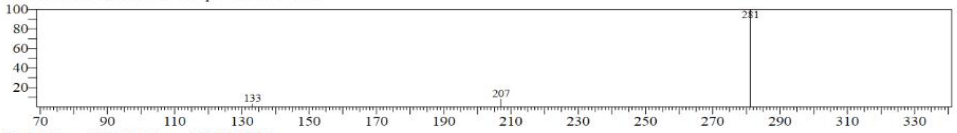
Hit#:1 Entry:212030 Library:WILEY8.LIB
SI:79 Formula:C17H26OS CAS:112303-78-3 MolWeight:278 RetIndex:0
CompName:BENZENEBUTANETHIOIC ACID, .ALPHA.,.ALPHA.,2,4,6-PENTAMETHYL-, S-ETHYL ESTER



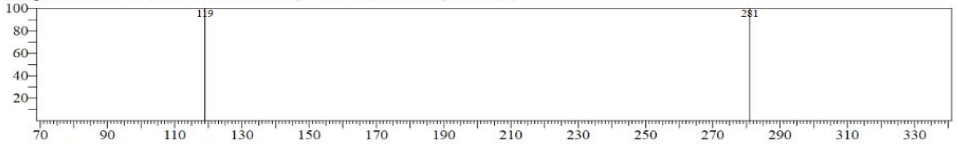
Hit#:2 Entry:211731 Library:WILEY8.LIB
SI:79 Formula:C16H22O2S CAS:0-00-0 MolWeight:278 RetIndex:0
CompName:(S)-1,1-DIMETHYLETHYL 3-HYDROXY-2-METHYL-5-PHENYL-4-PENTENEDITHIOATE



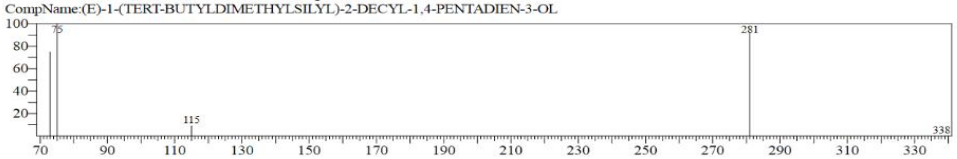
<< Target >>
Line#:11 R.Time:39.325(Scan#:7066) MassPeaks:3
RawMode:Averaged 39.320-39.330(7065-7067) BasePeak:281.15(684)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan



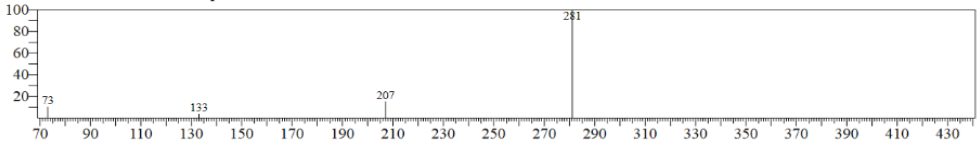
Hit#:1 Entry:215993 Library:WILEY8.LIB
SI:95 Formula:C19H23NO CAS:0-00-0 MolWeight:281 RetIndex:0
CompName:1-CYCLOHEXYL-5-METHYL-3-(P-TOLYLCARBONYL)PYRROLE



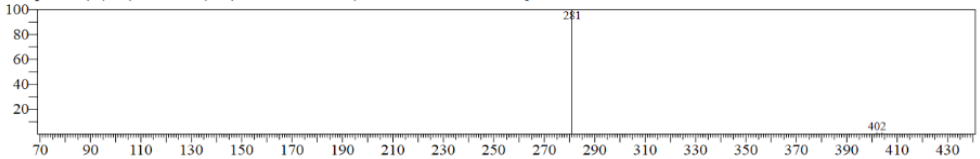
Hit#:2 Entry:283316 Library:WILEY8.LIB
SI:95 Formula:C21H42OSi CAS:0-00-0 MolWeight:338 RetIndex:0
CompName:(E)-1-(TERT-BUTYLDIMETHYLSILYL)-2-DECYL-1,4-PENTADIEN-3-OL



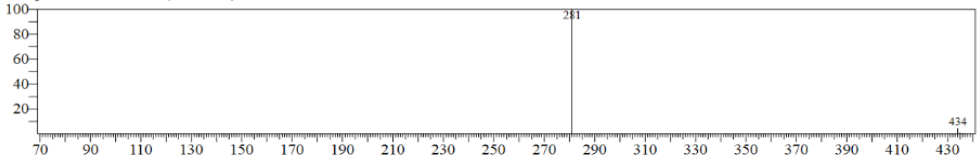
<< Target >>
Line#:12 R.Time:39.415(Scan#:7084) MassPeaks:4
RawMode:Averaged 39.410-39.420(7083-7085) BasePeak:281.15(1046)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan



Hit#:1 Entry:335947 Library:WILEY8.LIB
SI:86 Formula:C19H15BrO5 CAS:0-00-0 MolWeight:402 RetIndex:0
CompName:(+-)-1-(ACETOXY)-2-(1-BROMOETHYL)-3-METHOXYANTHRAQUINONE



Hit#:2 Entry:353737 Library:WILEY8.LIB
SI:85 Formula:C12H11F9O3SSi CAS:0-00-0 MolWeight:434 RetIndex:0
CompName:DIMETHYL(PHENYL)SILYL NONAFLUOROBUTANESULFONATE



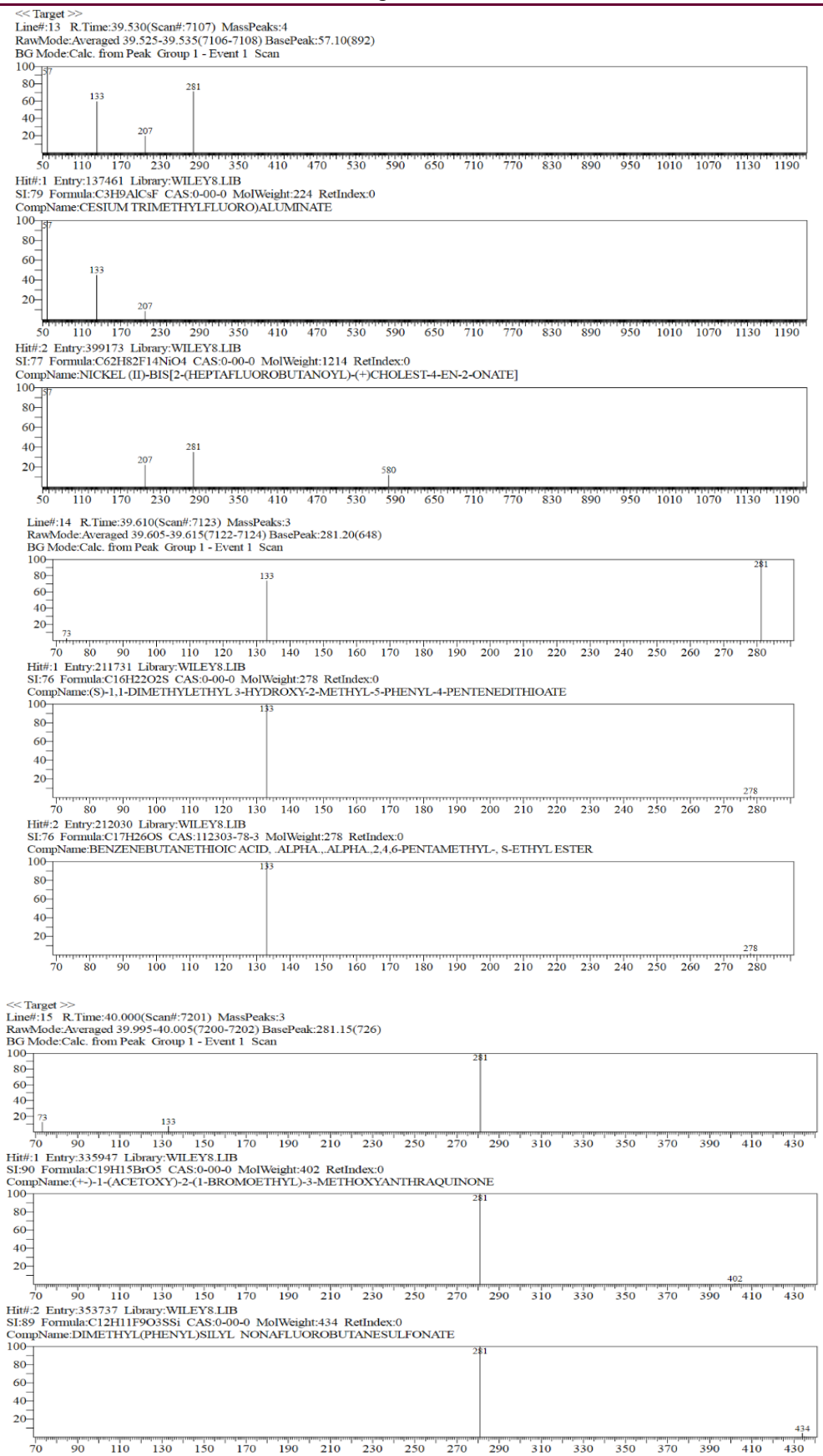


Figure 9: Specifications of all the compounds as a result of GCMS

Testing on Eggs

Figure 10: Analysis of drug over country eggs with developed blood vessels

Drug Testing on Eggs

At first, we maintained the country eggs in an incubator for upto 2 weeks in order to adapt the environment. After that, we induced our drug concentration via syringe, composed of *Khejri* leaves by drilling a little hole on the eggs. And, then the treatment procedure continued with our natural compound composed of *Khejri* leaves and added Doxorubicin [14], a standard procedure to proceed for upto 2 weeks, accompanied by the evaluation of anticancer activity. Then, screening of eggs for cancer gene expression- In control and Test samples treated groups were seen. It is seen via pictures that the blood vessel forms using the sample.

For More clarity

Group 1- 3 eggs	Untreated
Group 2- 3 eggs	Treatment with 1000ppm DMBA
Group 3- 3 eggs	Treatment with 1000ppm DMBA in addition with natural compound- *Khejri* leaves– Concentration 1
Group 4- 3 eggs	Treatment with 1000ppm DMBA in addition with natural compound- *Khejri* leaves- Concentration 2
Group 5- 3 eggs	Treatment with 1000ppm DMBA in addition with Doxorubicin (50uM/100mg/L)
Conclusion	Formation of blood vessels visible after 46 days

DISCUSSION

The authors demonstrated and designed the entire experiment in order to find the anti-cancerous values of phytochemicals present in the sample- *Khejri* Leaves. To begin with, the authors picked the toposheet of Bikaner, Rajasthan from Survey of India and studied the seasonal variations of different seasons of vegetation via satellite data so provided by Indian Space Research Organisation, India. Thereafter, sampling trip was conducted along with GPS tracker to find the desired location of tree/s there in Bikaner. Samples were collected easily and stored in laboratory.

The authors performed FTIR- Fourier Transfer Infrared Spectroscopy to find the functional group/s where the different peak falls.

After that, UV Spectroscopy was carried out to find the absorbance rate, along with FESEM EDAX that gives the elemental mineral composition so present in the sample.

After that, GCMS and HPLC were performed to find out what all compounds are there with their chemical structure and molecular formula that reflects that few of the compounds like Benzene, (1-Bromoethyl)- (Alpha-Bromoethyl) Benzene (1-

Bromoethyl) Benzene (Alpha-Bromoeth), Chloromethyl Chloroacetate, 1,2-Benzenedicarboxylic Acid, Diisooctyl Ester $$ Bis (6-Methylheptyl) Phthalate, 1,2-Benzenedicarboxylic Acid, Dioctyl Ester, 1,2-Benzenedicarbonic Acid, Dioctyl Ester, Phthalic Acid, 4-Nitrophenyl Octyl Ester, (E,E)-2- [3-(Dimethylamino)- 2-Propylidene] Cycloheptanone, D-Gluco-Heptonic Acid, 2,6-Anhydro-3-Deoxy-, Methyl Ester $$ Methyl .Beta.- (2,6-Anhydro-3-Deoxy-D-Arabi, Cesium Trimethylfluoro) Aluminate, 1,1-Dimethylethyl 3-Hydroxy-2-Methyl-5-Phenyl-4- Pentenedithioate, Nickel (Ii)-Bis[2-(Heptafluorobutanoyl)-(+)Cholest-4-En-2-ONATE] were present in the sample and are a look alike of anti-cancer medication. These compounds are having different importance that is not only confined to cancer but too to other disabilities/ diseases. Furthermore, they are combined with phytochemicals present in them that are bioactive and have toxicity which further reflects the medicinal importance present in the sample/s.

Proceeded with Blood cancer for *in-vitro* experimentation, cell lines of blood cancer were given treatment with our sample in different concentrations and we found that *Khejri* leaves are an anti-cancer agent which simply means that we can use it in the treatment of blood cancer.

As for further instance, we gave this sample treatment to eggs and as a result, the blood vessels form once the egg was fertilized which itself proves that yes, *Khejri* is having anti-cancerous properties which can be used as in a form of pellet or vaccine or drug etc whether Ayurvedic, homoeopathy, allopathy or Unani.

CONCLUSION

Investigation reveals that the extraction of *Prosopis Cineraria* (leaves) possessed significant anticancer activity that was analysed by GCMS and HPLC analysis and further tested on eggs further reveals the presence of phytoconstituents including squalene, steroids, tannins, ethers, phenolics and acids with respect to biological activities that further gestures the development of blood vessels over Eggs via inducing the sterilized drug obtained from *Khejri* leaves. The presence of various bioactive compounds indicates the potential in treating many infectious/ malignant diseases. It is a good medicinal alternative to human health.

Data Availability: The authors confirm that the data supporting the findings of this study are available within this article.

Acknowledgments: Thankful to Tribiotech Research Lab, Bishop Heber College, St Joseph College, Trichy, Tamil Nadu, for getting the tests done under their payable facility for other university scholars.

Credit authorship contribution statement: Yamini and SR Singara Subramanian designed performed experiments, and wrote the manuscript. All authors are reviewed in the original article.

Abbreviations

MTT- 3-4, 5 dimethylthiazol-2yl-2, 5-diphenyl tetrazolium bromide

FTIR- Fourier transform infrared spectroscopy

GCMS- Gas chromatography Mass spectroscopy

IC50- Inhibitory Concentration at 50%

HPLC- High performance liquid chromatography

UVS- Ultra Violet Spectroscopy

FESEM- Field Emission Scanning Electron Microscope

EDAX- Energy Dispersive X-ray Spectroscopy

DMSO- Dimethyl Sulfoxide

FBS- Fetal Bovine Serum

CO_2- Carbon Dioxide

DMEM- Dulbecco's modified Eagle medium

PBS- Phosphate Buffer Saline

MOLT 4- Human T lymphoblast, acute lymphoblastic leukaemia (cell line slides)

OD- Optical density

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