GC MS analysis of Phytochemical compounds present in the leaves of *Euphorbia prostrata* Ait.

V. Sundara Prabha[1], S.Beschi Antony Rayan[2]
[1]*P G, Department of Botany, St. Xavier's College, Palayamkottai - 627002*
[2]*Research Department of Botany, St. Xavier's College, Palayamkottai - 627002*

Abstract- **The pharmaceutical industries are looking for the presence of secondary metabolites as the lead compounds to develop new drugs. Phytochemical analysis and identification of compounds is an important step in the entire process of drug discovery from the plants which have been used traditionally to cure ailments. One of such medicinal plants which has been used traditionally is Euphorbia prostrata Ait., is subjected to GCMS analysis. The plants were shade dried and extracted with ethanol using fine powder of the plant. The GCMS analysis showed the presence of six compounds based on the data base of National Institute Standard and Technology (NIST) and Wiley spectra Libraries. Further studies on the biological activity of these compounds may shed more light on the medicinal importance of this plant.**

1. INTRODUCTION

The pharmaceutical industries are looking for the plant derivatives as the raw material for new drugs. The plants which have been traditionally used as the curing agents have been screened for the presence of biologically active compounds throughout the world. The presence of secondary metabolites makes it as a medicinally important one. Majority of the biologically active compounds are secondary metabolites only. Discoveries of early drugs like cocaine, codine, digitoxin etc are coming from plant resources (Newman *et al*., 2000; Butler, 2004). Drug discovery process from medicinal plants begins with collection and identification of plants of interest, phytochemical analysis and identification of compounds, their biological activity and using them as lead molecules for developing new drugs. One of the key step in the entire process is phytochemical screening of the medicinal plants which have been used traditionally to cure ailments.

The genus *Euphorbia* is the largest genus of medicinal plants. *E. prostrata* is used in fever, abdominal disorder and as blood purifier in many parts of the world. *Euphorbia prostrata* is a reputed medicinal plant serving as the source of drug in the Indian System of Medicine and used in treatment of many diseases of skin, digestive system, anti-asthmatic, anti-diabetic etc. The extract of *E. prostrata* has been found to have significant anti-inflammatory, analgesic, hemostatic (stops bleeding) and wound healing properties (Single and Pathak, 1989)

Euphorbia prostrata is a reputed medicinal plant serving as the source of drug in the Indian System of Medicine and used in treatment of many diseases of skin, digestive system, anti-asthmatic, anti-diabetic etc. It is also used in treating eye canker and to prepare antiseptic paste. Even though preliminary works and phytochemical analysis have been done on this plant in other regions of the world, phytochemical analysis using high throughput techniques have not been done especially for a local population of this plant. Hence the present project was has been carried out to identify the phytoconstituents using GC-MS analysis.

2. MATERIALS AND METHODS PLANT MATERIAL

The selected plant, *Euphorbi prostrata* Ait. belonging to the family Euphorbiacea was collected from the coastal areas of Kanyakumari district of Tamilnadu, India. *Euphorbia prostrata* Ait. (Euphorbiaceae) is a small annual herb found all over India especially in foot hills of Himalayas. It is native to the West Indies and certain parts of South America and also widely naturalized in many other parts of the world. These

are branched, prostrate with many stems spreading from the roots, slender up to 20 cm long; leaves green but occasionally purplish red. The oval-shaped leaves are up to 1.0 cm long with finely toothed edges. The inflorescence is a cyathium, less than 2 mm wide, with white petal-like appendages surrounding the actual flowers. There are four male flowers and a single female flower, the latter developing into a lobed, hairy fruit 1.0 – 2.0 mm wide.

Fresh plant were collected from sandy coasts of Kanyakumari district and washed properly with distilled water. The leaves were shade dried at room temperature. Dried leaves were uniformly pulverised using mechanical grinder. The ethanol extract was prepared with 25.0 g of powdered plant material using 150 ml of ethanol with soxhlet apparatus and it was used for the following tests and analysis.

PHYTOCHEMICAL ANALYSIS USING GCMS

1. Sample Preparation for GCMS Analysis:

A. *Collection And Processing Of Plant Material*

Fresh plants of *euphorbia prostrata* Ait. were collected from the coastal areas of Kanyakumari district, Tamil Nadu, India. The plants were washed thoroughly in running water to remove soil particles and dusts and finally washed with sterile distilled water.

B. *Plant Sample Extraction*

10.0 g of shade dried, powdered plant sample was extracted with 200 ml ethanol using a Soxhlet apparatus and filtered through ash less filter paper with sodium sulphate (2.0 g). The extract was concentrated to 1.0 ml by bubbling nitrogen into the solution

2. GC-MS Analysis

GC-MS analysis was performed using The JEOL GCMATE II GC-MS with data system is a high resolution, double focusing instrument.[Maximum resolution: 6000 Maximum calibrated mass: 1500 Daltons equipped with an Elite-5MS (5% diphenyl/95% dimethyl poly siloxane) fused a capillary column (30 × 0.25 µm ID × 0.25 µm df)]. For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 2 µl was employed (a split ratio of 10:1). The injector temperature was maintained at 250 °C, the ion-source temperature was 200 °C, the oven temperature was programmed from 110 °C (isothermal for 2 min), with an increase of 10 °C/min to 200°C, then 5 °C/min to 280°C, ending with a 9 min isothermal at 280 °C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 2 min, and the total GC/MS running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas.

3. IDENTIFICATION OF COMPONENTS

Interpretation of mass spectrum GCMS was conducted using data base of National Institute Standard and Technology (NIST) and Wiley spectra Libraries. The compound prediction is based on Dr. Duke's Phytochemical and Ethnobotanical Databases by Dr. JimDuke of the Agricultural Research Service/USDA.

Interpretation of GC-MS was conducted using the database of the spectrum of known components stored in the NIST Library. The molecular weight, molecular formula and the number of hits used to identify the name of the compound from NIST and Wiley spectra Libraries were recorded.

4. RESULTS AND DISCUSSION GC-MS ANALYSIS

The results pertaining to GC-MS analysis leads to the identification of number of compounds from the GC fractions of the ethanolic extract of *E.prostrata*. These compounds were identified through mass spectrometry attached with GC. The results of the present study are tabulated.

The heights of the peak indicate the relative concentrations of the components present in the sample. The mass spectrometer analyzes the compounds eluted at different times and percentage of area occurred to identify the nature and structure of the compounds.

The presence of nine major peaks (Figure 1) show that there are nine major phytoconstituents which are characterized and identified using NIST library. The retention times (RT) of compounds are given in

minutes (Figure 2). The nine major compounds identified according to the active functional groups with their retention time (RT) present in the constituents are 17-(1,5-dimethylhexyl)-10,13-dimethyl 3-styryl hexadecahydrocyclopenta(a)phenanthrene-2-one,2-Cyclopentene-1-undecanoic acid methyl ester;9-Octadecenoic acid methyl ester;6- tridecenoic acid-13-(2-cyclopentene-1-yl); 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl);3,8,12-tri-O-acetoxy7-desoxyingol-7-one;
2-Cyclopentene-1-tridecanoicacid; Hexadecanoic acid and octadecanoic acid 9 10-dichloro- methyl ester(Table 2).

In addition, many compounds also having anti-cancer, anti-oxidant, anti-tumor, anti-inflammatory, ant androgenic, dermatitigenic, hypocholesterolemic, 5-Alpha reductase inhibitor. GCMS analysis shows that the plant contains many phytoconstituents with high biological activities. Further investigation may shed more light on how this plant and its new metabolites could be explored in the pharmaceutical industry.

Figure 1. GCMS chromatogram of ethanolic extract of Euphorbia prostrata

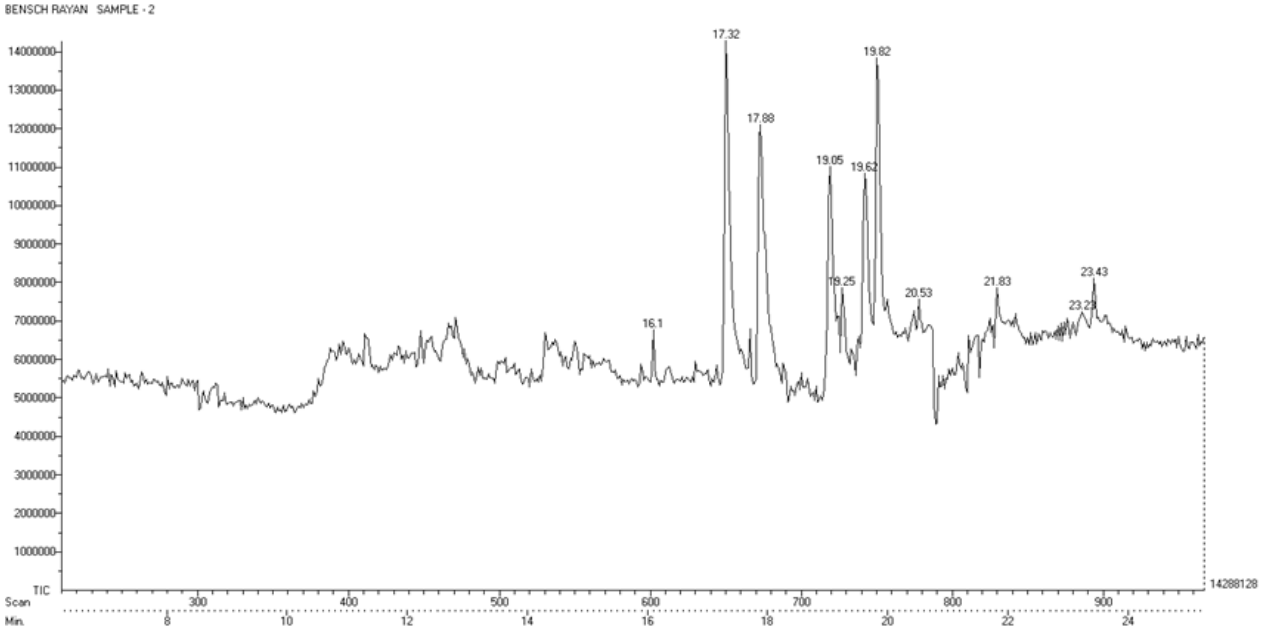
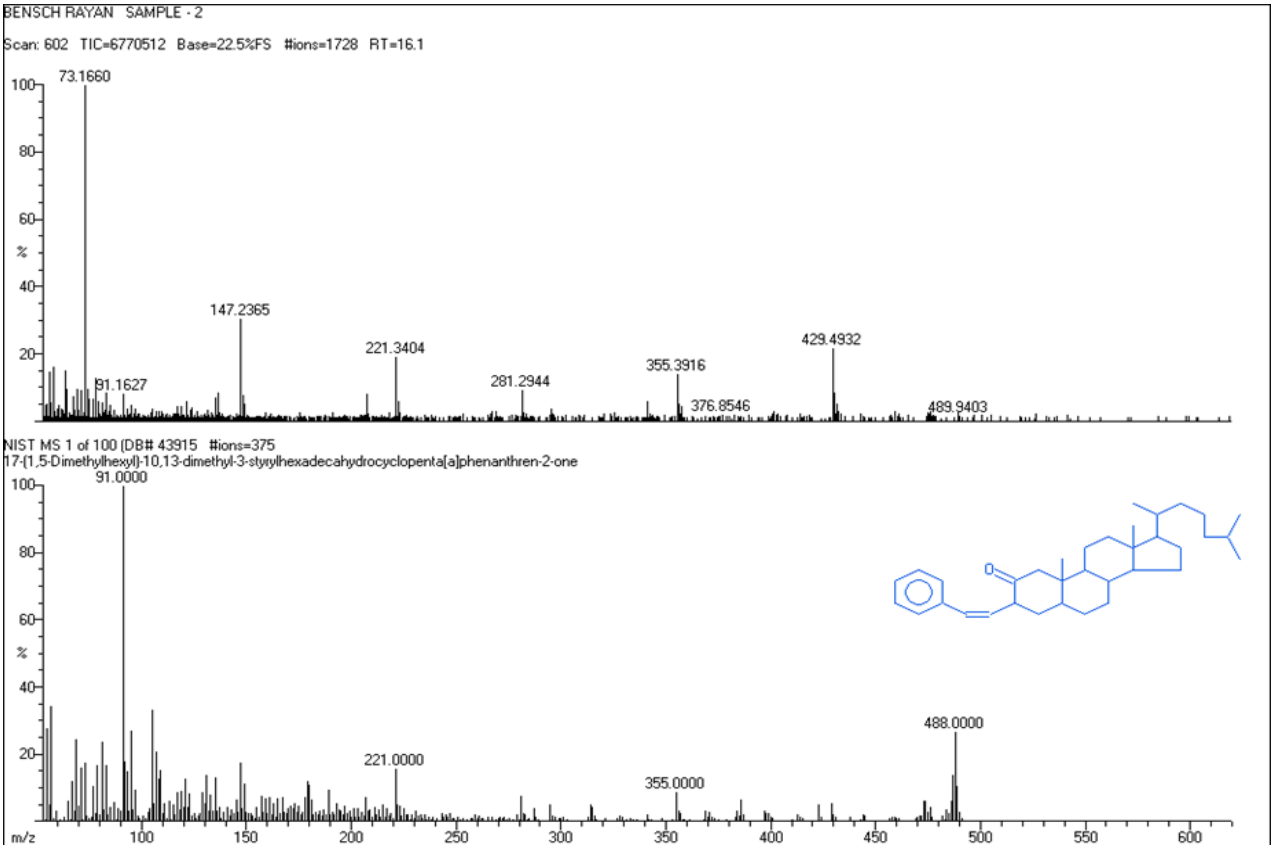


Table 2: Phytoconstituents identified by GC MS analysis.

Compound names	Molecular formula	Molecular weight	Retention number
3,8,12-tri-O-acetoxy7-desoxyingol-7-one	$C_{26}H_{34}O_9$	490.542	23.43
2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)	$C_{19}H_{32}O_2$	294.26	21.83
2-Cyclopentene-1-tridecanoicacid	$C_{18}H_{32}O_2$	280.445	19.82
6- tridecenoic acid-13-(2-cyclopentene-1-yl);	$C_{18}H_{30}O_2$	278.429	19.62
octadecanoic acid 9 10-dichloro- methyl ester.	$C_{19}H_{36}Cl_2O_2$	367.393	19.25
9-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.487	19.05
2-Cyclopentene-1-undecanoic acid methyl ester	$C_{17}H_{30}O_2$	266.418	17.88
Hexadecanoic acid	$C_{16}H_{32}O_2$	256.424	17.33
17-(1,5-dimethylhexyl)-10,13-dimethyl 3-styryl hexa decahydrocyclopenta(a) phenanthrene-2-one	$C_{32}H_{52}O$	488.79	16.1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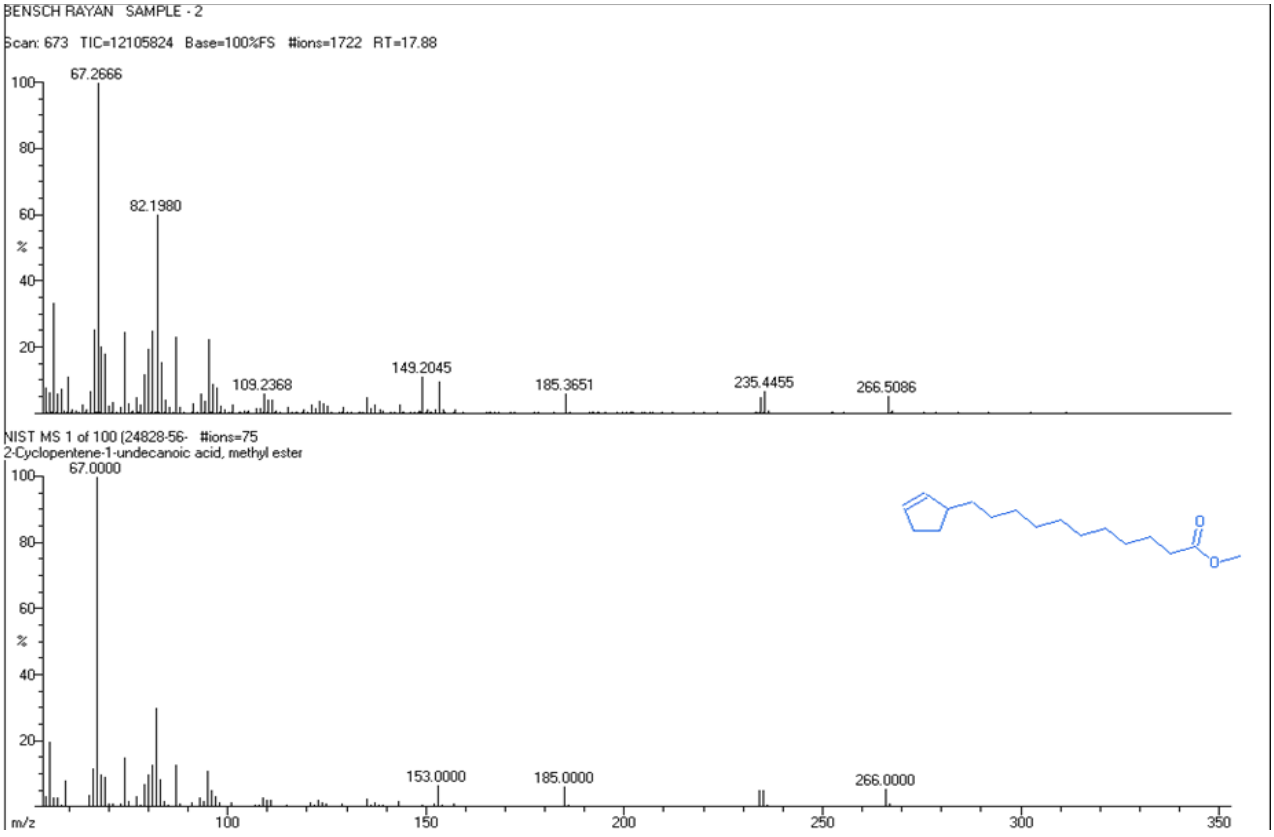
91.1627
221.3404
281.2944
355.3916
376.8546
429.4932
489.9403
NIST MS 1 of 100 (DB# 43915 #ions=375
17-(1,5-Dimethylhexyl)-10,13-dimethyl-3-styrylhexadecahydrocyclopenta[a]phenanthren-2-one
91.0000
221.0000
355.0000
488.0000
m/z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Scan: 673 TIC=12105824 Base=100%FS #ions=1722 RT=17.88
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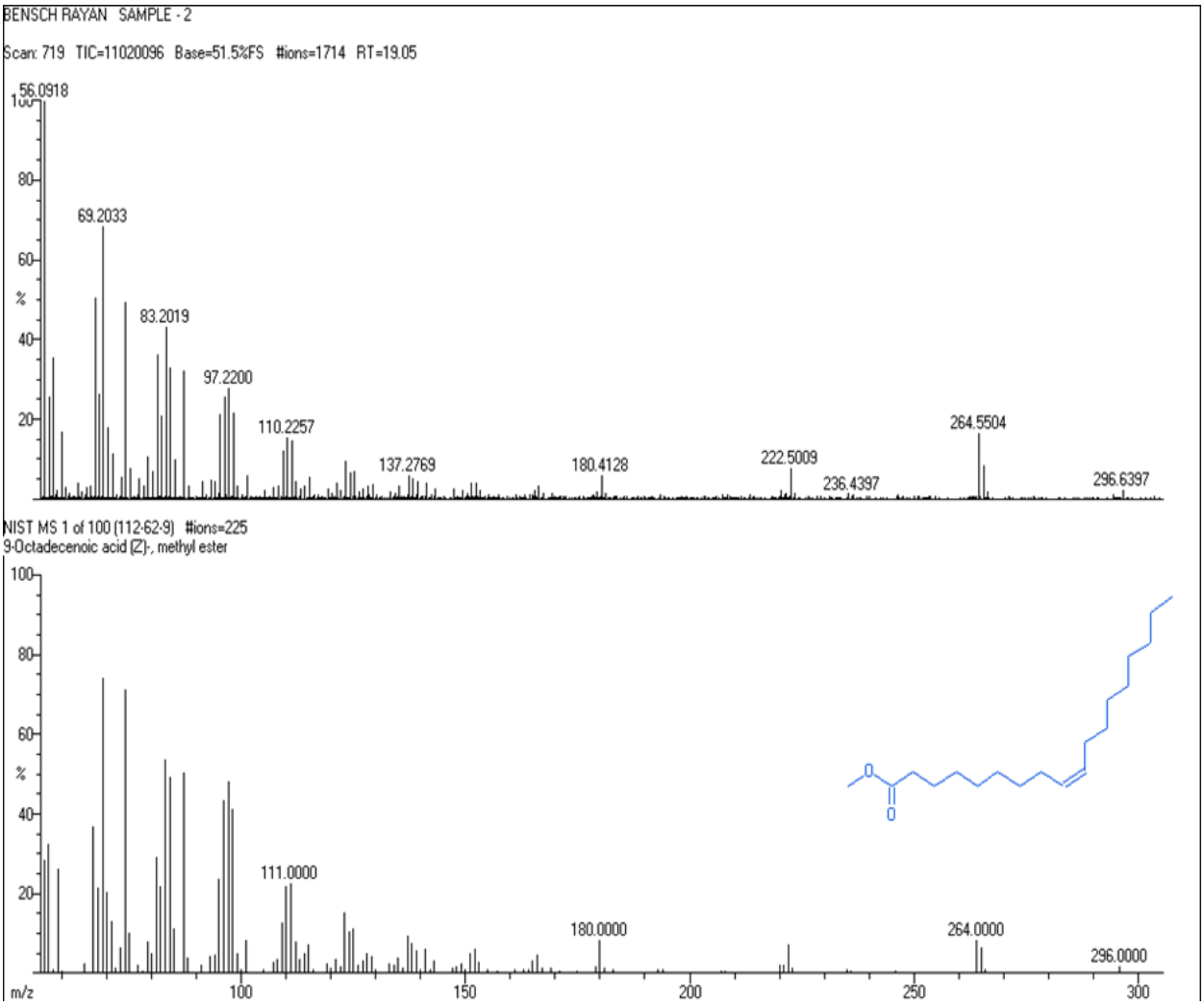
82.1980
109.2368
149.2045
185.3651
235.4455
266.5086
NIST MS 1 of 100 (24828-56- #ions=75
2-Cyclopentene-1-undecanoic acid, methyl ester
67.0000
153.0000
185.0000
266.0000
m/z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Scan: 719 TIC=11020096 Base=51.5%FS #ions=1714 RT=19.05
56.0918
69.2033
83.2019
97.2200
110.2257
137.2769
180.4128
222.5009
236.4397
264.5504
296.6397
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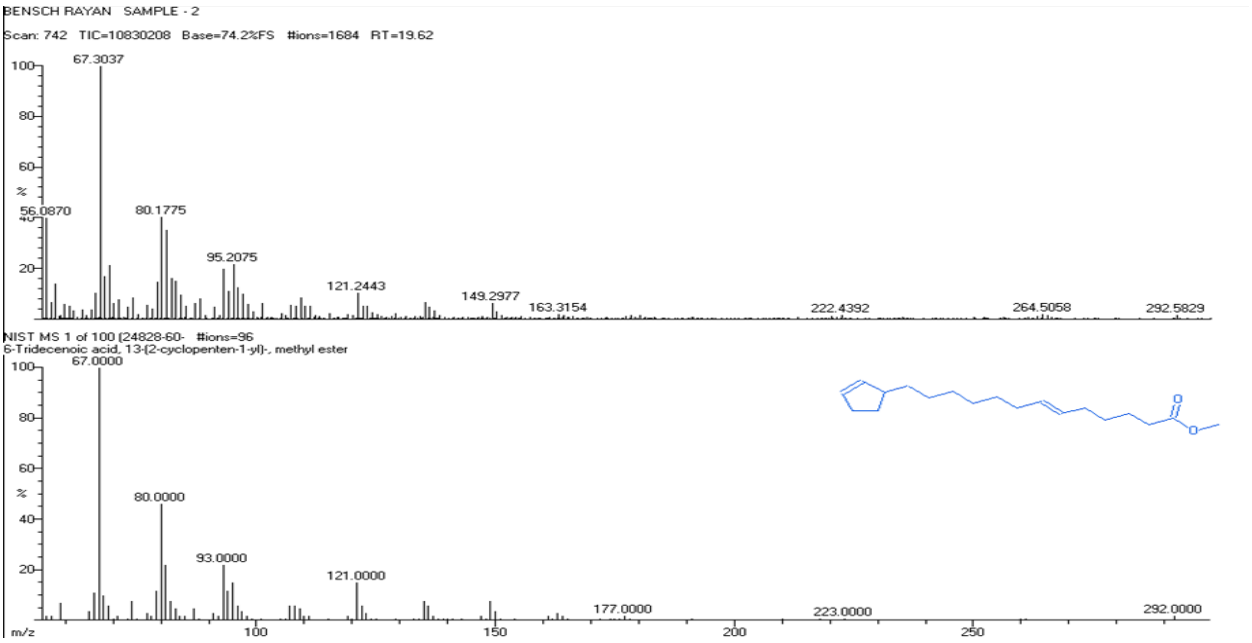
111.0000
180.0000
264.0000
296.0000
m/z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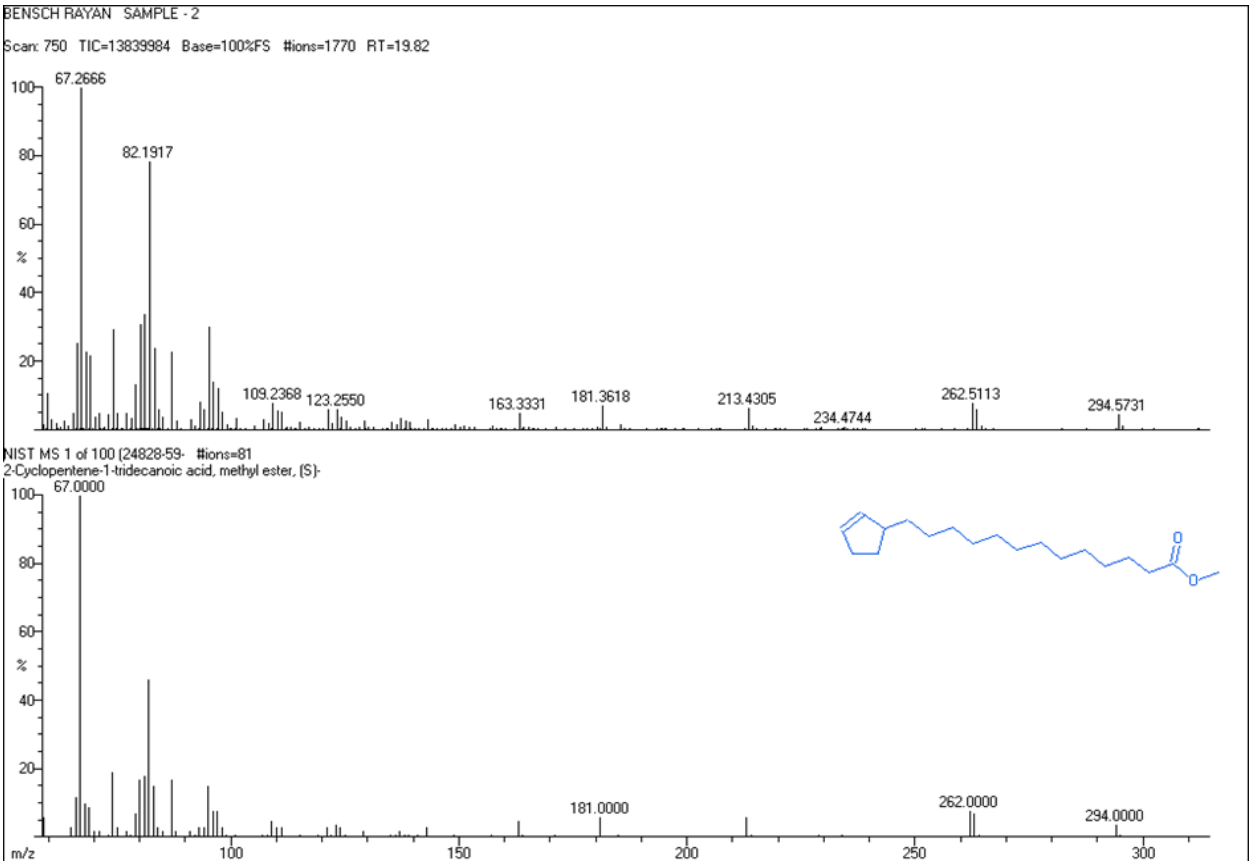
56.0870
80.1775
95.2075
121.2443
149.2977
163.3154
222.4392
264.5058
292.5829
NIST MS 1 of 100 (24828-60- #ions=96
6-Tridecenoic acid, 13-(2-cyclopenten-1-yl)-, methyl ester
67.0000
80.0000
93.0000
121.0000
177.0000
223.0000
292.0000
m/z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163.3331
181.3618
213.4305
234.4744
262.5113
294.5731
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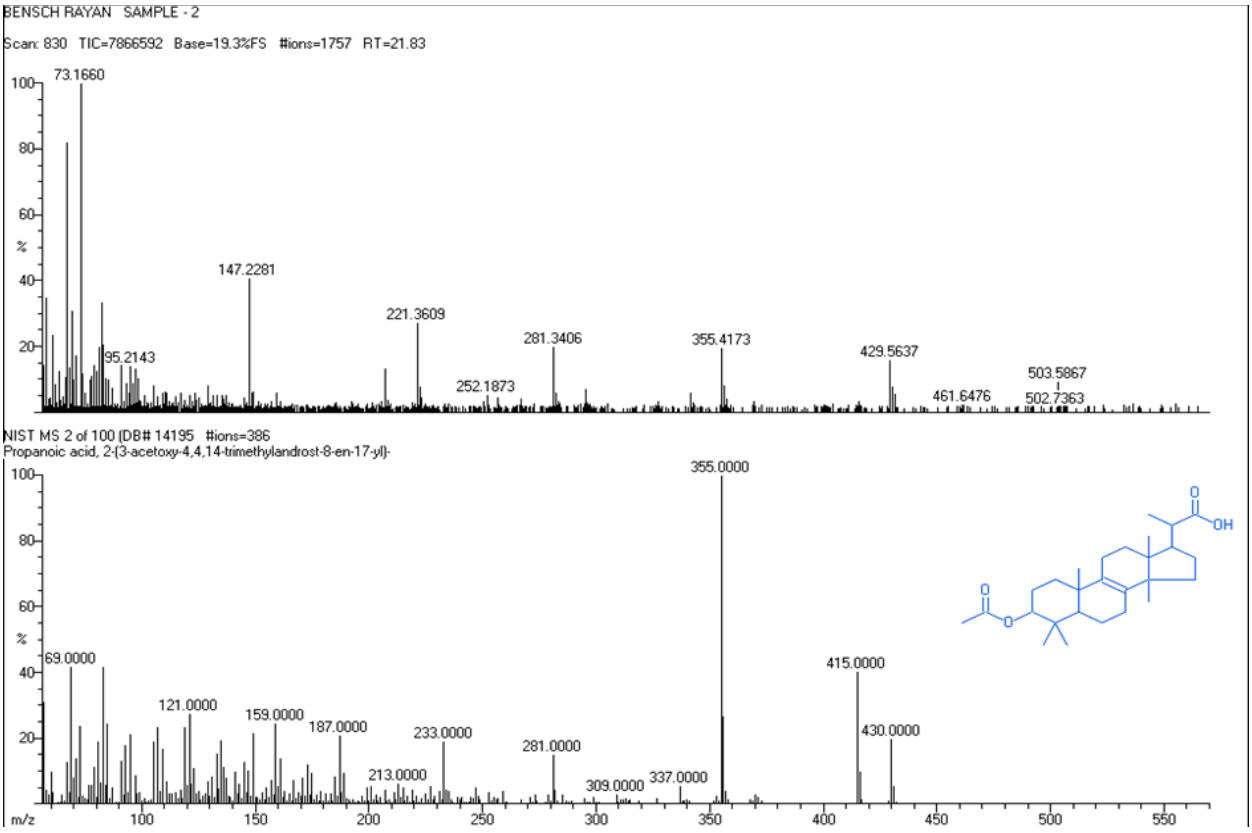
2-Cyclopentene-1-tridecanoic acid, methyl ester, (S)-
67.0000
181.0000
262.0000
294.0000
m/z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95.2143
252.1873
281.3406
355.4173
429.5637
461.6476
503.5867
502.7363
NIST MS 2 of 100 (DB# 14195 #ions=386
Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-
355.0000
69.0000
121.0000
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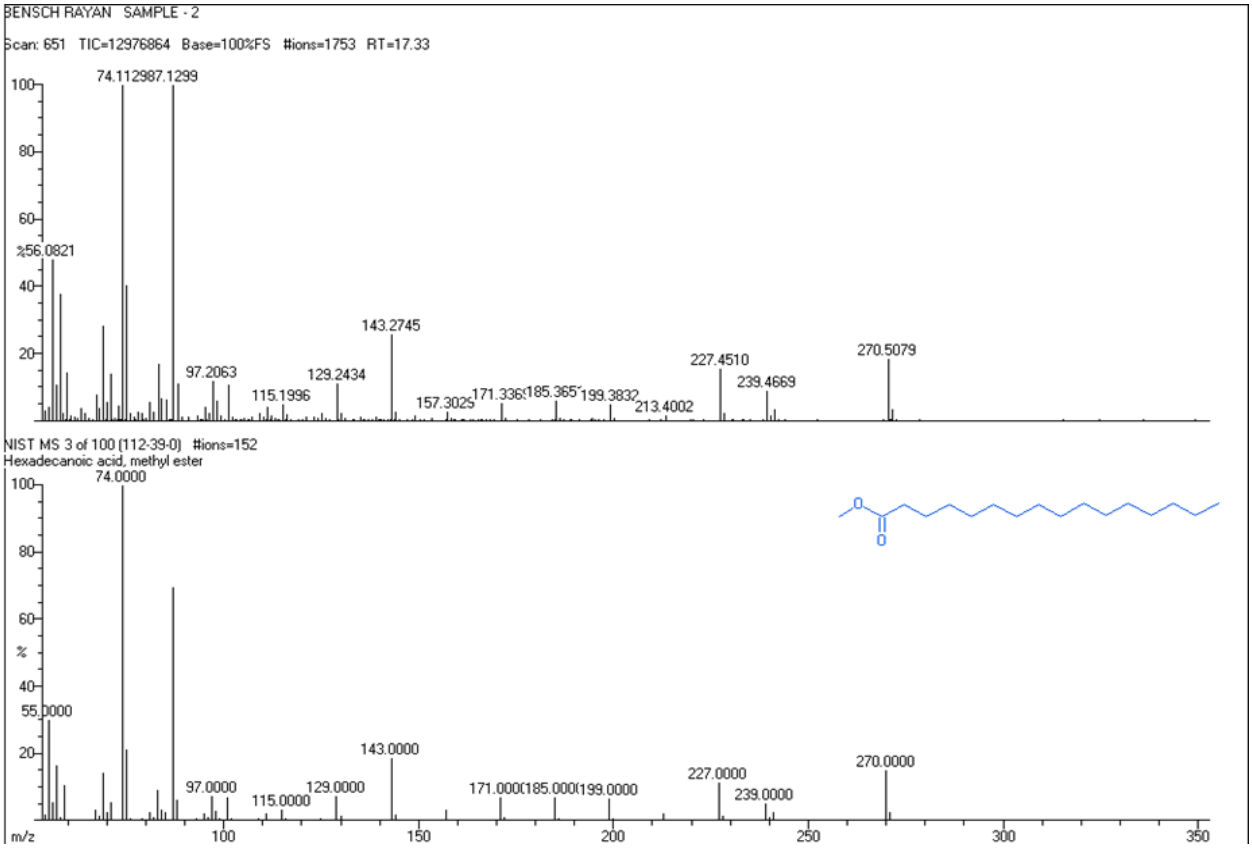
187.0000
233.0000
213.0000
281.0000
309.0000
337.0000
415.0000
430.0000
m/z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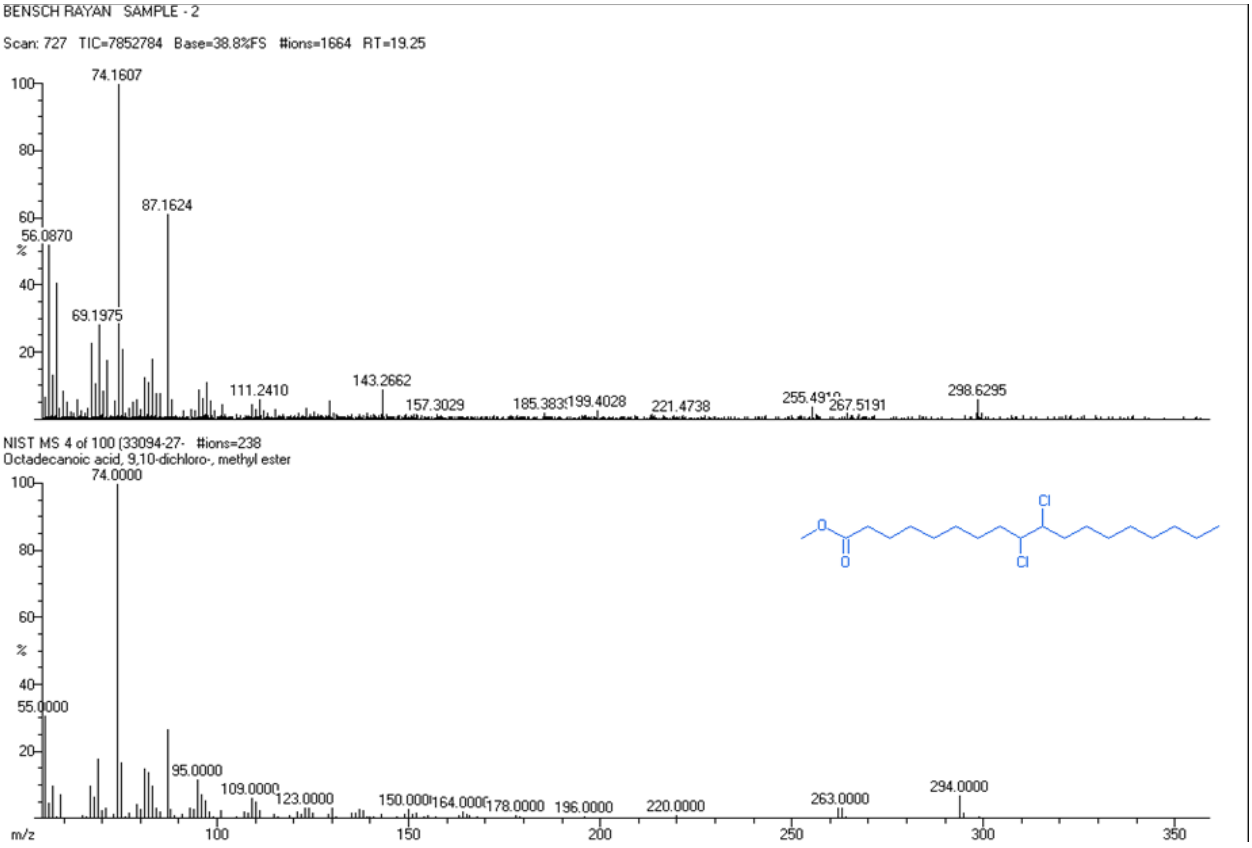
74.112987.1299
%56.0821
143.2745
97.2063
129.2434
115.1996
157.3025
171.336
185.365
199.3832
213.4002
227.4510
239.4669
270.5079
NIST MS 3 of 100 (112-39-0) #ions=152
Hexadecanoic acid, methyl ester
74.0000
55.0000
143.0000
97.0000
129.0000
115.0000
171.000
185.000
199.0000
227.0000
239.0000
270.0000
m/z


BENSCH RAYAN SAMPLE - 2
Scan: 727 TIC=7852784 Base=38.8%FS #ions=1664 RT=19.25
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87.1624
56.0870
69.1975
111.2410
143.2662
157.3029
185.383
199.4028
221.4738
255.49
267.5191
298.6295
NIST MS 4 of 100 (33094-27- #ions=238
Octadecanoic acid, 9,10-dichloro-, methyl ester
74.0000
55.0000
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109.0000
123.0000
150.000
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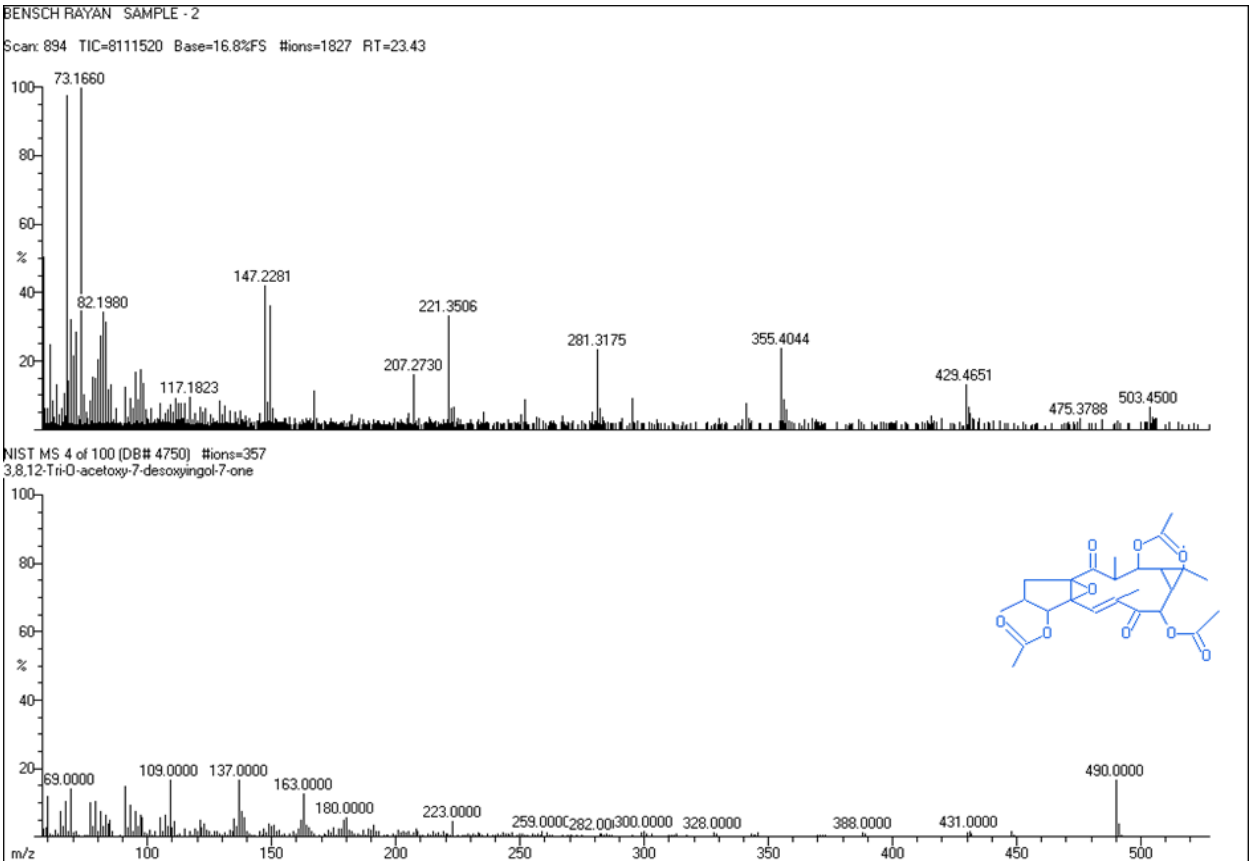
196.0000
220.0000
263.0000
294.0000
m/z



CONCLUSION

The extract of E. prostrata has been reported to have significant anti-inflammatory, analgesic, haemostatic (stops bleeding) and wound healing properties (Singla and Pathak, 1989)

Euphorbia prostrata is a reputed medicinal plant serving as the source of drug in the Indian System of Medicine and used in treatment of many diseases of skin, digestive system, anti-asthmatic, anti-diabetic etc. It is also used in treating eye canker and to prepare antiseptic paste. Ethanolic extracts was subjected to phytochemical screening and identifying the phytoconstituents by GCMS method. GCMS analysis showed that nine major phytoconstituents with the ethanolic extract.

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