

Identification of Bioactive Compounds Present in the Crude Methanolic Leaf Extract of *Acalypha Fruticosa* Forssk. through GC-MS Analysis

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ABSTRACT

The aim of the study is to characterize the bioactive chemical compounds present in crude methanolic leaf extract of *Acalypha fruticosa*. Qualitative determination of the different biologically active compounds from crude extracts of *A. fruticosa* using Gas Chromatography–Mass Spectrometry (GC-MS) revealed 15 types of high and low molecular weight chemical entities with varying quantities present in the leaf extracts. These chemical compounds are considered biologically and pharmacologically important. Thus, identification of different biologically active compounds in the methanolic leaf extracts of *A. fruticosa* warrants further biological and pharmacological studies.

Keywords *Acalypha fruticosa*; Euphorbiaceae; crude extract; bioactive compounds.

Medicinal plants are considered as rich resources of ingredients which can be used in drug development either pharmacopoeial, non-pharmacopoeial or synthetic drugs. It plays an appreciable role in the development of human cultures around the whole world. Moreover, some plants are considered as important source of nutrition and as a result of that they are recommended for their therapeutic values (Rooksarr *et al.*, 2017). Isolation of natural products from plants is still a challenging task. Undoubtedly hybrid methods like LC-NMR or GC-MS made on-line structure elucidation possible and provided impressive examples of natural product identification without prior isolation (Ammar *et al.*, 2017).

Acalypha fruticosa Forssk. belongs to the family Euphorbiaceae inhabiting the foot hills of Western Ghats of Coimbatore and Erode districts, Tamil Nadu, India. The leaves are prescribed for digestive troubles such as dyspepsia, colic and diarrhea. The root portion is used in Siddha system of medicine for curing gonorrhoea (Pullaiah, 2006). Boiled root decoction is used to cure cerebral malaria (Sahoo, 2001). Despite these huge medicinal values still the medical properties of this plant is unexplored yet. Hence, the present work was aimed at to identifying the therapeutic bioactive compounds from the study species, *Acalypha*

fruticosa.

MATERIALS AND METHODS

Collection of plant materials

The clean and healthy leaves of the study species, *Acalypha fruticosa* were collected from Chennimalai (highly arid), Erode district and they were shade dried.

Preparation of plant extracts

To know the presence of major phytochemicals, the shade dried leaves of the population of Maruthamalai were made into a fine powder of 40 mesh size using the pulverizer. Following that, 100 g of the powder was filled in the filter paper and successively extracted using 500 ml solvents *viz.* petroleum ether, hexane, chloroform and finally with methanol using the soxhlet extractor for 8 – 10 hours (Gafner *et al.*, 1985). The extract was filtered through Whatman No.1 filter paper to remove all undissolved matter, including cellular materials and other constitutions that are insoluble in the extraction solvents.

Gas Chromatography - Mass Spectrometry (GC-MS) (Joachim and Hübschmann, 2008)

Gas chromatography (GC) analysis was carried out at South Indian Textile Research Institute (SITRA), Coimbatore. It is one of the key techniques generally used for screening/ identification of many groups of plant phytochemicals. The high attainable separation power in combination with wide range of the detectors employing various detection principles to which it can be coupled makes GC an important, often irreplaceable tool in the analysis at trace level of plant phytochemical compounds. Gas chromatographical study includes the important optimization process such as i) introduction of sample extract onto the GC column, ii) separation of its components on an analytical column and iii) detection of target analysis by using mass spectrometric (MS) detector.

Table 1. Chemical components of the methanolic extract of the *Acalypha fruticosa* leaves using GC-MS analysis.

S.No	Component	Molecular formula	Retention time/min.	Molecular weight (m/z)
1.	Benzene, Ethyl (CAS)-	C ₈ H ₁₀	2.230	106
2.	Lycorenan-7-One,1,8,9,10- Trimethoxy-1-Methyl-	C ₁₉ H ₂₅ O ₅ N	4.111	347
3.	Hexyl Tiglate	C ₁₁ H ₂₀ O ₂	7.643	184
4.	2H- Indeno1,2-B Oxirene Octahydro-1A.Alpha 1B.B-	C ₉ H ₁₄ O	8.223	138
5.	Aromadendrene	C ₁₅ H ₂₄	10.034	204
6.	1,5 Diazacyclododecan-6-One-	C ₁₀ H ₂₀ ON ₂	10.784	184
7.	Phenol, 2,4,-Bis(1,1- Dimethyl ethyl)	C ₁₄ H ₂₂ O	12.506	206
8.	1-Dotriocantonal	C ₃₂ H ₆₆ O	13.987	466
9.	Butenoic acid 3,7 dimethyl 6-octenyl ester	C ₁₄ H ₂₄ O ₂	14.086	224
10.	Vitamin A Aldehyde	C ₂₀ H ₂₈ O	18.809	284
11.	7, 9 -Di -Tert-Butyl-1-Oxaspiro [4,5] Deca – 6,9 Diene – 2,5-DI	C ₁₇ H ₂₄ O ₃	19.680	276
12.	Cyclopenten-1-one, 4, hydroxyl – 3 methyl -2-(2,4-Pentadienyl)	C ₁₁ H ₁₄ O ₂	19.980	178
13.	Citronellyl propionate	C ₁₃ H ₂₄ O ₂	22.100	212
14.	Piperidine,1-(2-Phenylethyl)-(CAS) -	C ₁₃ H ₁₉ N	23.121	189
15.	1-Dodecene	C ₁₂ H ₂₄	25.263	168

GC-MS analysis

Five ml of methanol extract was evaporated to dryness and reconstituted in 2 µl methanol. The extracts were then subjected to GC-MS analysis. Chromatographic separation was carried out with CE GC 8000 top MSMD 8000 Fyson instrument with Db 35 nr column (10 m x 0.5 mm, 0.25 im film thickness). Heating programs were executed from 100 - 250 °C at 3 minutes by using the helium was used as a carrier gas with a flow rate of 1 ml/min in the split mode (1:50). An aliquot (2 il) of oil was injected into the column with the injector heater at 250 °C.

Analytical conditions

Injection temperature at 250°C, interface temperature at 200°C, quadruple temperature at 150°C and ion source temperature at 230°C were maintained. Injection was performed in split less mode.

Data analysis

The mass spectra of compounds in samples were obtained by electron ionization (EI) at 70 eV, and the detector operated in scan mode from 20 to 600 atomic mass units (amu). Identifications were based on the molecular structure, molecular mass and calculated fragmentations. Resolved spectra were identified for phytochemicals by using the standard mass spectral database of WILEY and NIST.

RESULTS AND DISCUSSION

The methanol extracts of the leaves of *A. fruticosa* were obtained from dry habitat of Chennimalai (highly arid) and subjected to GC-MS analysis. The phytochemical components with their retention time (RT/min.), molecular formula and molecular weight (*m/z*) are presented in Table 1 and Figs. 1 - 15. The analysis by using GC-MS technique revealed the presence of following fifteen compounds: One compound of phenolic group, Phenol, 2,4,-Bis(1,1-Dimethyl ethyl), one compound of aldehyde group, Vitamin A aldehyde, one compound of alkane group, Cyclopenten-1-one, 4, hydroxyl - 3 methyl -2-(2,4-Pentadienyl), five compounds of alkaloid group, 2H-Indeno12-B Oxirene Octahydro-1A.Alpha 1B.B-, Lycorenan-7-One,1,8,9,10- Trimethoxy-1-Methyl-, Benzene Ethyl (CAS)-, Piperidine,1-(2-Phenylethyl)-(CAS) and quinone alkaloids of 1 - Dodecene, one compound of diterpenoid group, 7, 9 -Di -Tert-Butyl-1-Oxaspiro [4,5] Deca – 6,9 Diene – 2,5-DI, four compounds of saturated cyclic hydrocarbon group, Citronellyl propionate, Butenoic acid 3,7 dimethyl 6-octenyl ester, Hexyl Tiglate and 1,5 Diazacyclododecan-6-One, one compound of sesquiterpenoids group, Aromadendrene and one compound of fatty alcohol, 1-Dotriocantonal. They are mainly the constituents of essential oils such as aromatic phenolic compounds, and a single

Sample ID: AND

Acquired on 18-Jul-2009 at 18:57:3E
Reverse fit factor [REV]: 491

EM-86 23 (2.230) Rf (3,6.000)

1.43e5

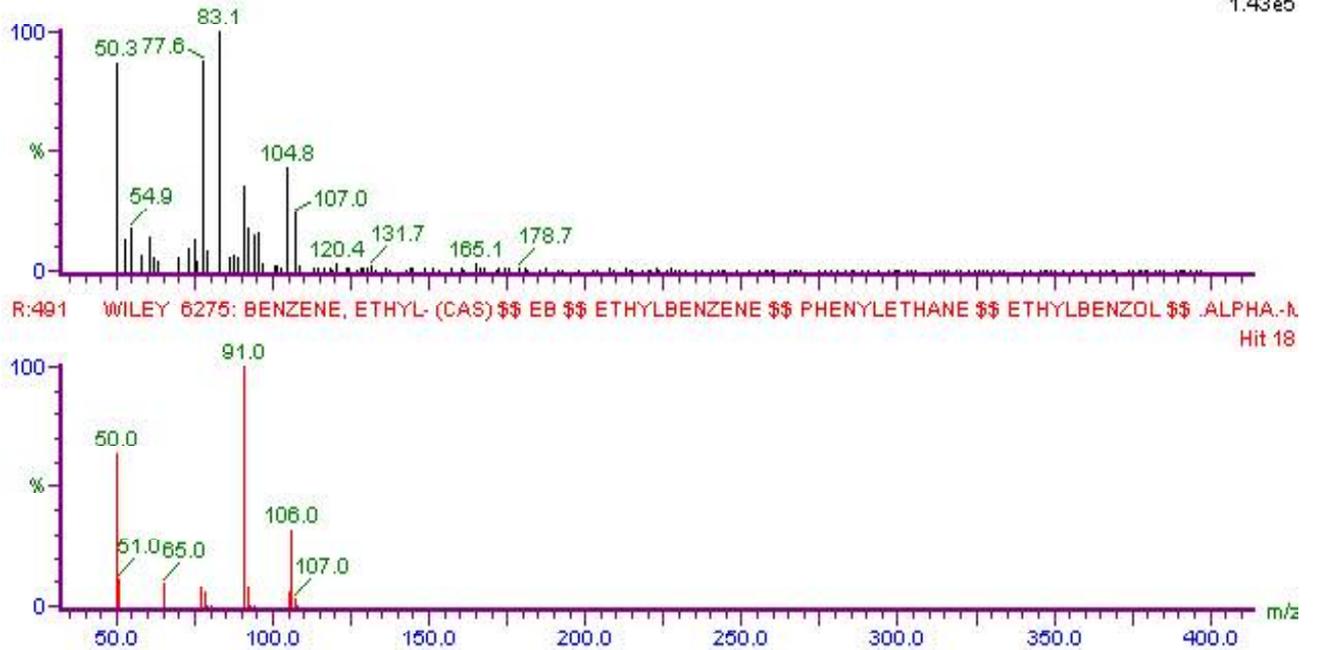


Fig. 1. Mass spectra of the methanolic extract component I of *Acalypha fruticosa* leaves of Chennimalai population.

Sample ID: AND

Acquired on 18-Jul-2009 at 18:57:3E
Reverse fit factor [REV]: 52E

EM-86 211 (4.111) Rf (3,6.000)

7.37e4

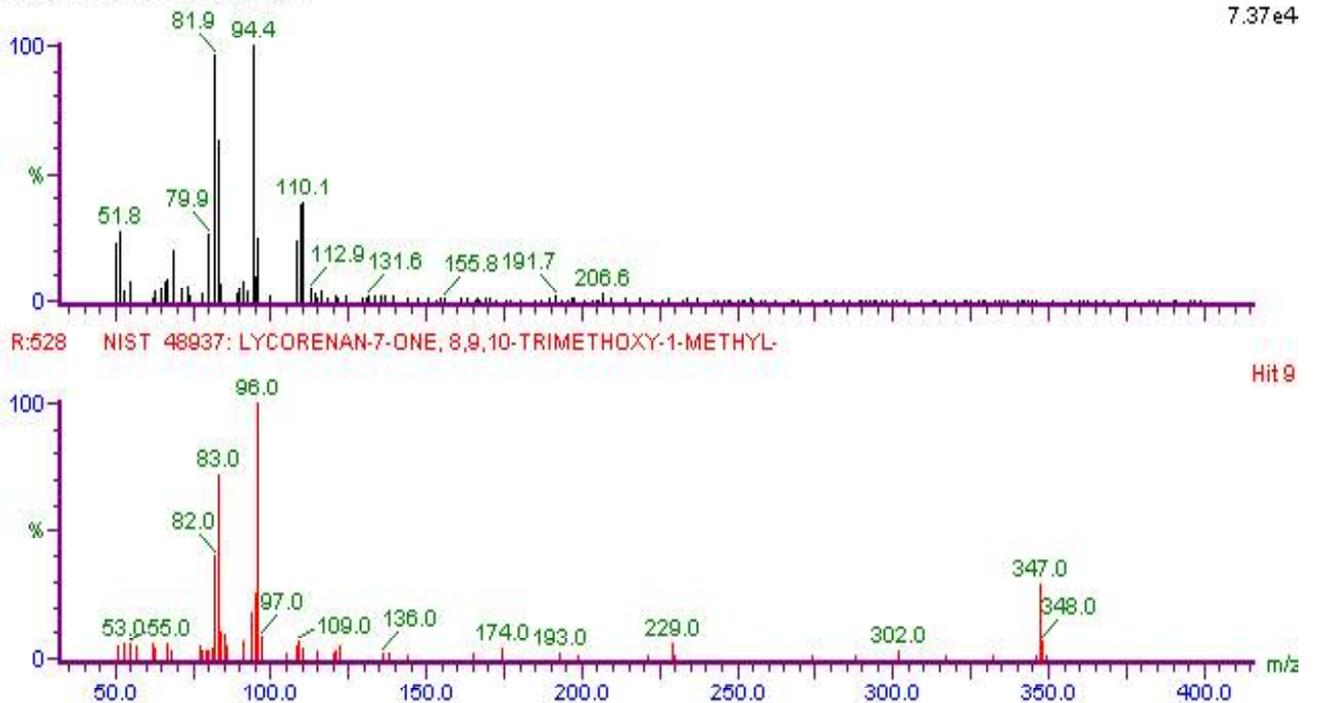


Fig. 2. Mass spectra of the methanolic extract component II of *Acalypha fruticosa* leaves of Chennimalai population.

Sample ID: AND

Acquired on 18-Jul-2009 at 18:57:3E
Reverse fit factor [REV]: 421

EM-86 564 (7.643) Rf (3,6.000)

4.27e5



R:421 WILEY 51858: HEXYL TIGLATE

Hit 18

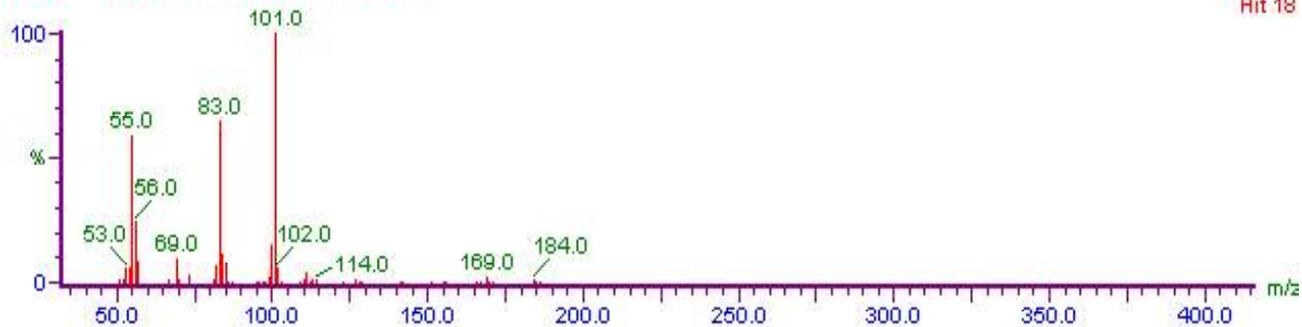


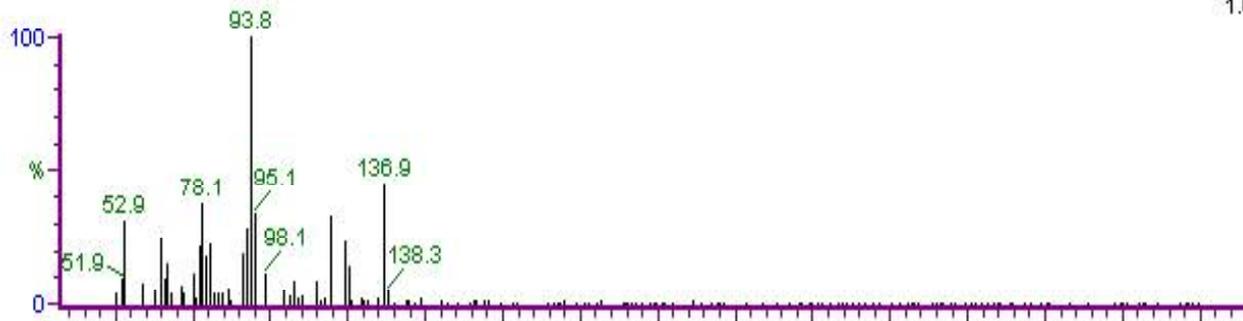
Fig. 3. Mass spectra of the methanolic extract component III of *Acalypha fruticosa* leaves of Chennimalai population.

Sample ID: AND

Acquired on 18-Jul-2009 at 18:57:3E
Reverse fit factor [REV]: 581

EM-86 622 (8.223) Rf (3,6.000)

1.03e5



R:581 NIST 6974: 2H-INDENO 1,2-B OXIRENE, OCTAHYDRO-, (1A.ALPHA.,1B.BETA.,5A.ALPHA.,6A.ALPHA.)

Hit 7

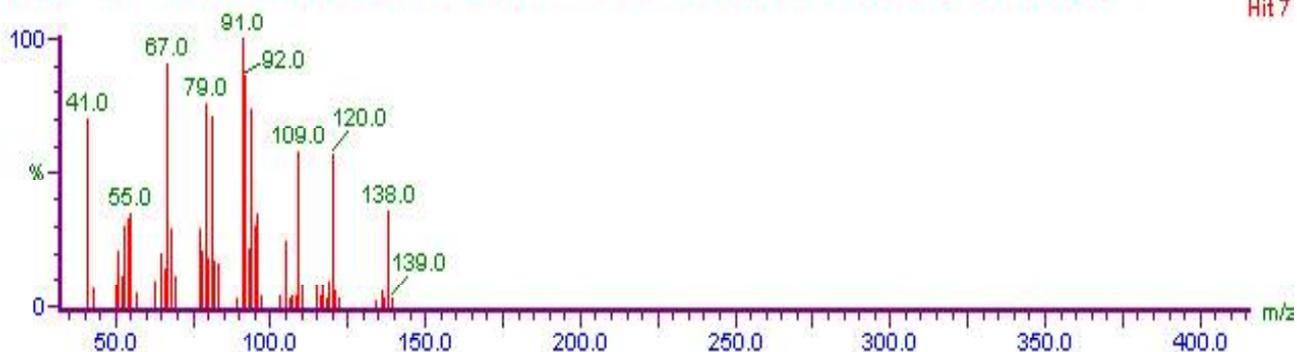


Fig. 4. Mass spectra of the methanolic extract component IV of *Acalypha fruticosa* leaves of Chennimalai population.

Sample ID: AND

Acquired on 18-Jul-2009 at 18:57:3E

Reverse fit factor [REV]: 81E

EM-86 803 (10.034) Rf (3,6.000)

5.72e5



R:818 WILEY 68476: AROMADENDRENE

Hit 2

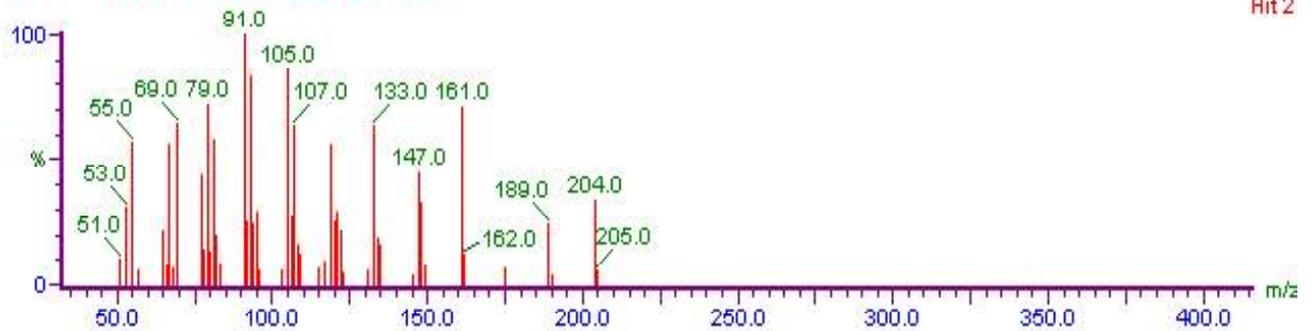


Fig. 5. Mass spectra of the methanolic extract component V of *Acalypha fruticosa* leaves of Chennimalai population.

Sample ID: AND

Acquired on 18-Jul-2009 at 18:57:3E

Reverse fit factor [REV]: 51E

EM-86 878 (10.784) Rf (3,6.000)

9.17e5



R:512 NIST 18839: 1,5-DIAZACYCLODODECAN-6-ONE

Hit 13

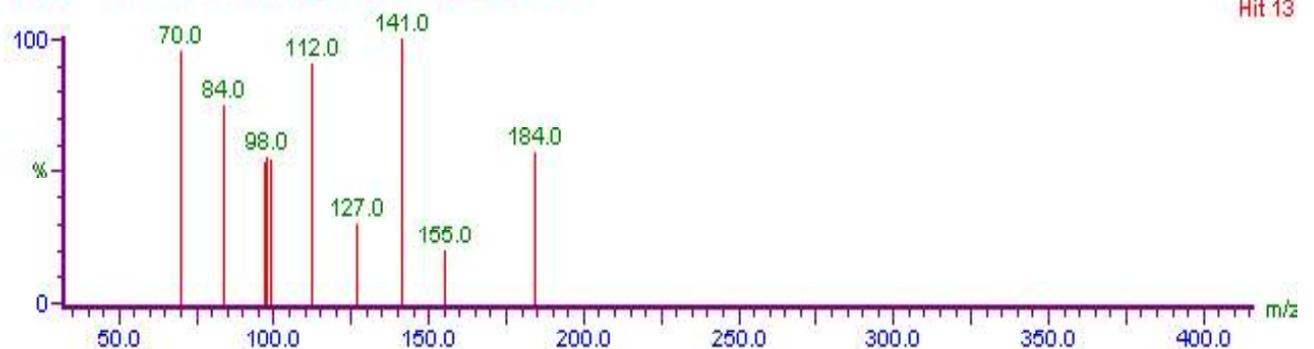


Fig. 6. Mass spectra of the methanolic extract component VI of *Acalypha fruticosa* leaves of Chennimalai population.

Sample ID: AND

Acquired on 25-Sep-2009 at 12:37:05
Reverse fit factor [REV]: 737

EM-225 1050 (12.506) Rf(3,6.000)

8.54e4

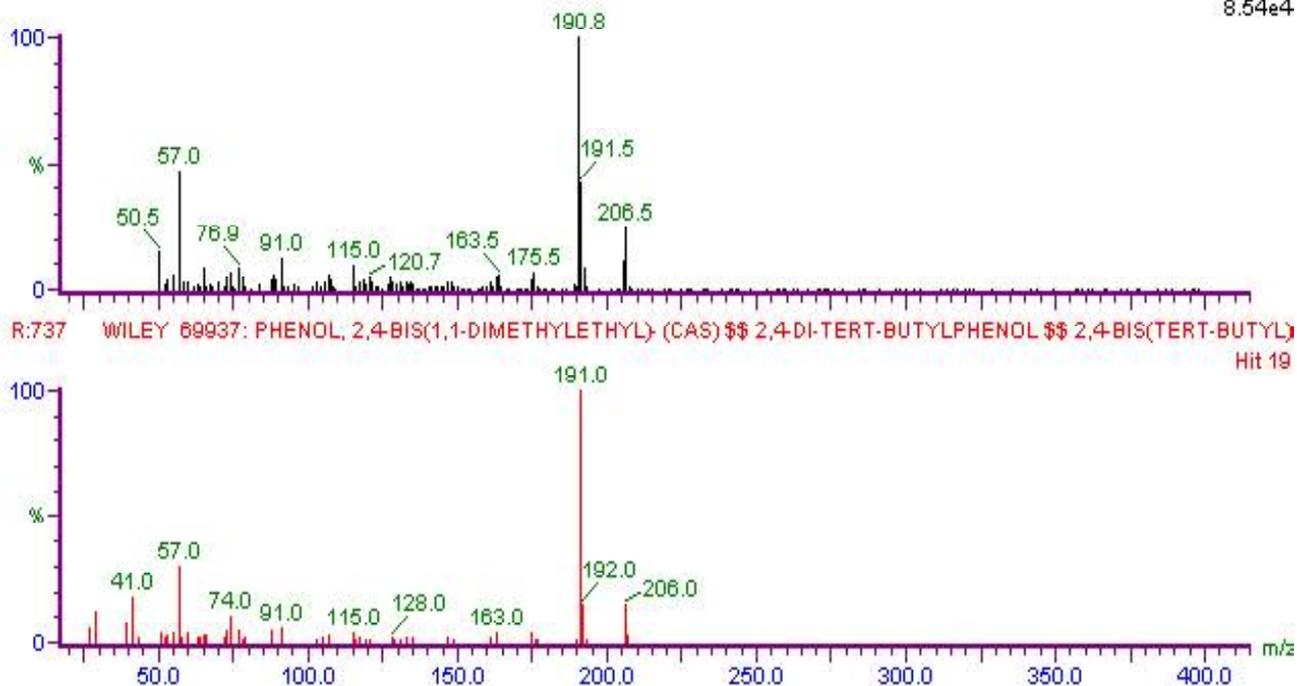


Fig. 7. Mass spectra of the methanolic extract component VII of *Acalypha fruticosa* leaves of Chennimalai population.

Sample ID: AND

Acquired on 25-Sep-2009 at 12:37:05
Reverse fit factor [REV]: 662

EM-225 1198 (13.987) Rf(3,6.000)

2.50e4

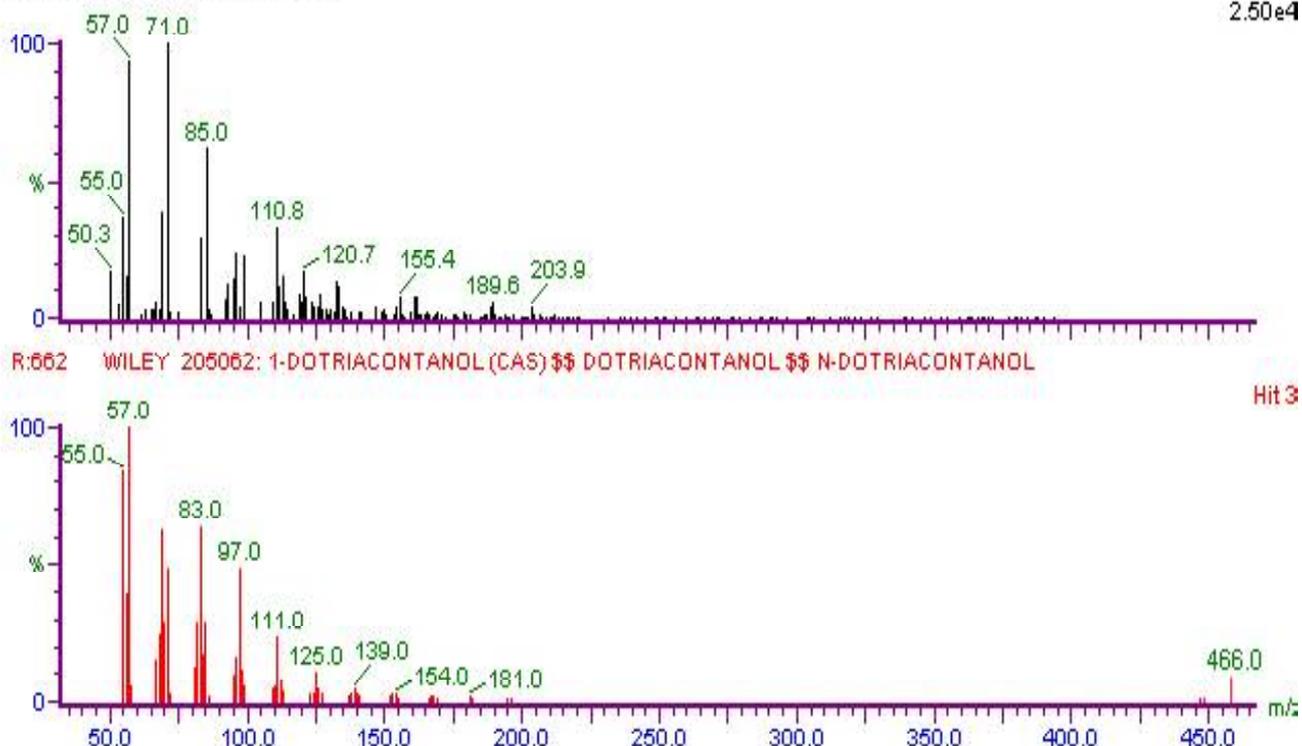


Fig. 8. Mass spectra of the methanolic extract component VIII of *Acalypha fruticosa* leaves of Chennimalai population.

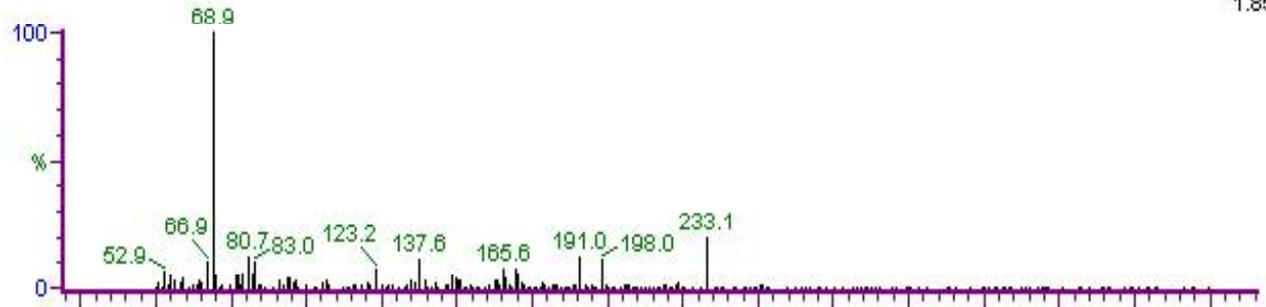
Sample ID: AND

Acquired on 18-Jul-2009 at 18:57:36

Reverse fit factor [REV]: 517

EM-96 1208 (14.086) Rf (3,6.000)

1.85e6



R:517 NIST 28685: BUTENOIC ACID, 3,7-DIMETHYL-6-OCTENYL ESTER

Hit 15

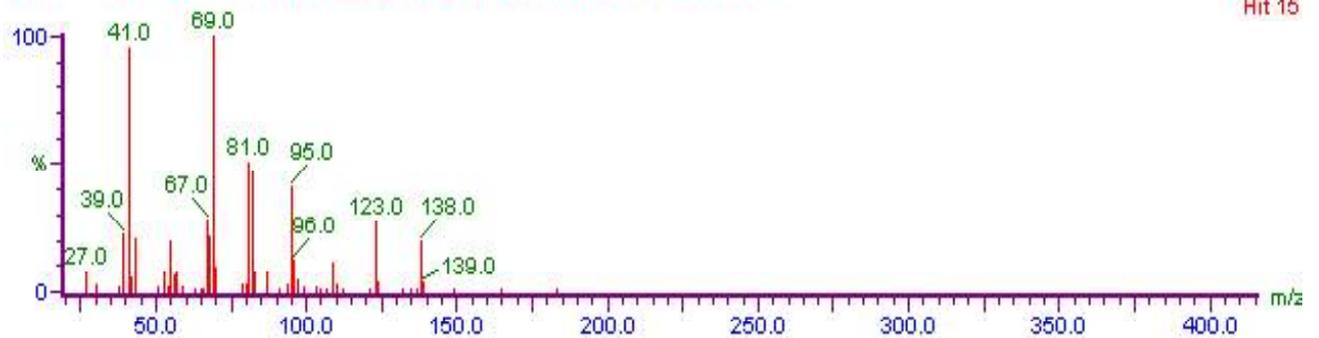


Fig. 9. Mass spectra of the methanolic extract component IX of *Acalypha fruticosa* leaves of Chennimalai population.

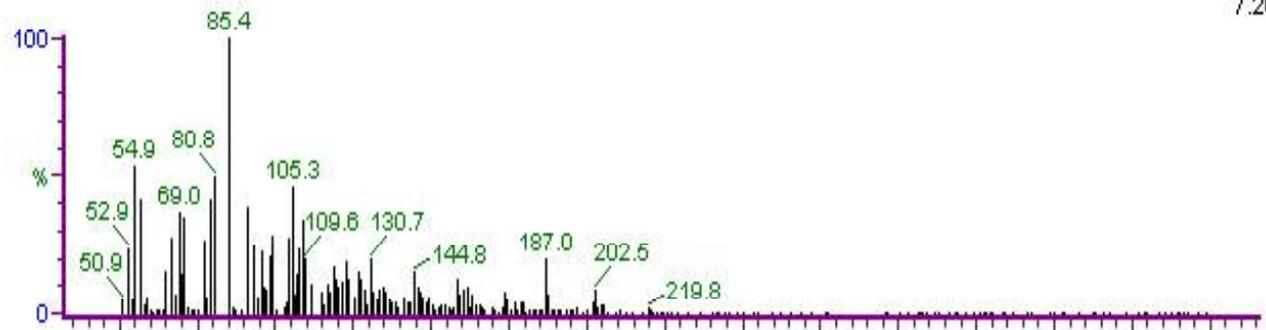
Sample ID: AND

Acquired on 25-Sep-2009 at 12:37:05

Reverse fit factor [REV]: 773

EM-225 1680 (18.809) Rf (3,6.000)

7.20e4



R:773 NIST 40247: VITAMIN A ALDEHYDE

Hit 10

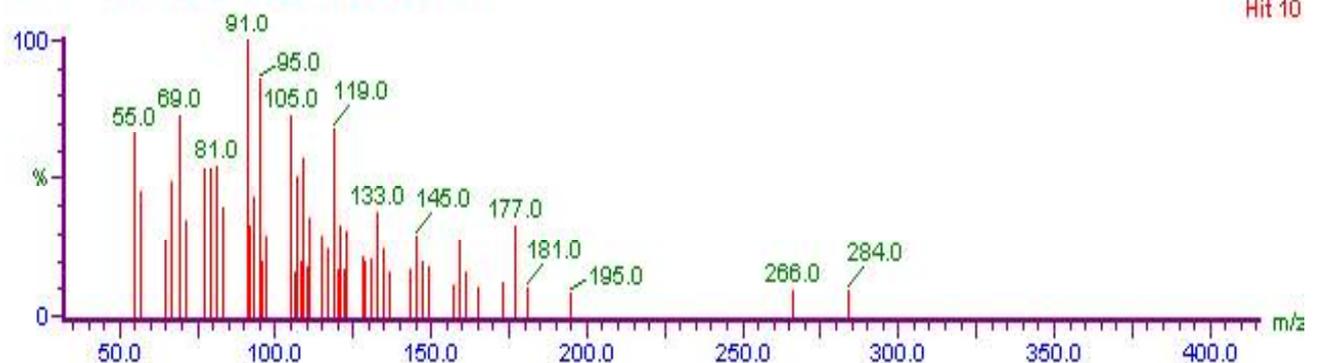


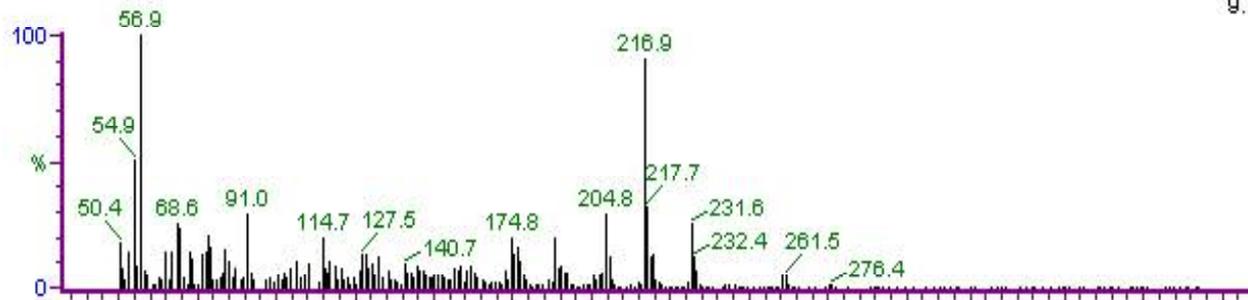
Fig. 10. Mass spectra of the methanolic extract component X of *Acalypha fruticosa* leaves of Chennimalai population.

Sample ID: AND

Acquired on 25-Sep-2009 at 12:37:05
Reverse fit factor [REV]: 735

EM-225 1767 (19.680) Rf (3,8.000)

9.74e4



R:735 WILEY 124993: 7,9-DI-TERT-BUTYL-1-OXASPIRO[4.5]DECA-6,9-DIENE-2,8-DIONE

Hit 3

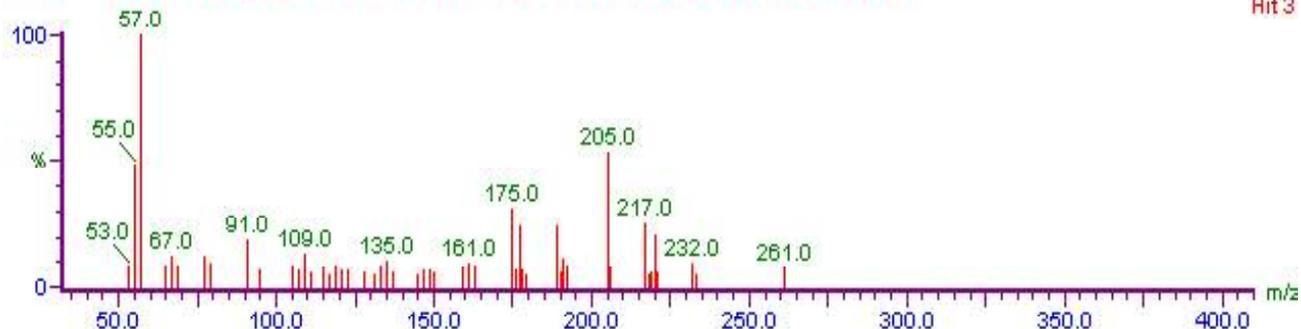


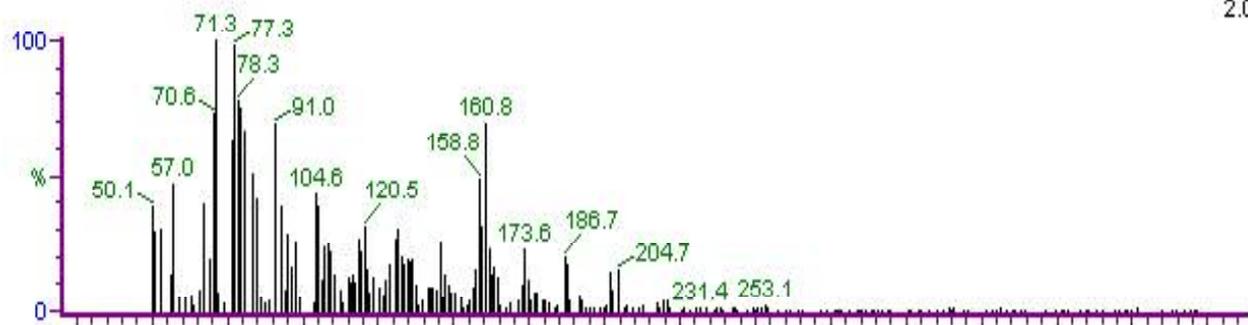
Fig. 11. Mass spectra of the methanolic extract component XI of *Acalypha fruticosa* leaves of Chennimalai population.

Sample ID: AND

Acquired on 25-Sep-2009 at 12:37:05
Reverse fit factor [REV]: 504

EM-225 1797 (19.980) Rf (3,8.000)

2.02e4



R:504 NIST 17259: 2-CYCLOPENTEN-1-ONE, 4-HYDROXY-3-METHYL-2-(2,4-PENTADIENYL)-, (Z)-(+)

Hit 17



Fig. 12. Mass spectra of the methanolic extract component XII of *Acalypha fruticosa* leaves of Chennimalai population.

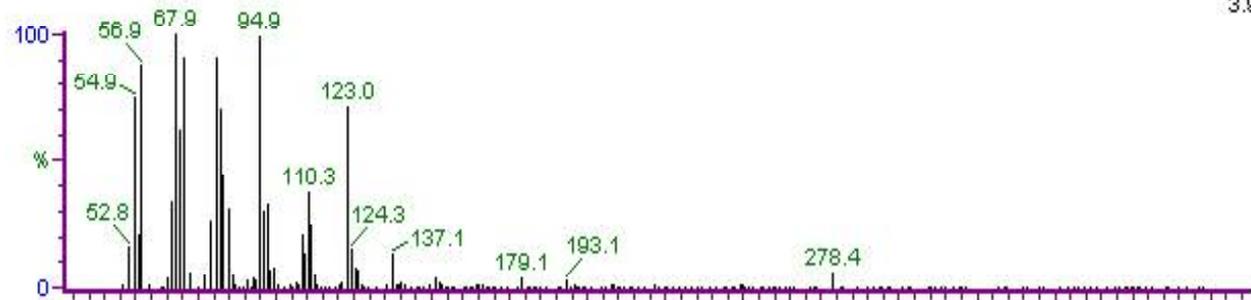
Sample ID: AND

Acquired on 18-Jul-2009 at 18:57:36

Reverse fit factor [REV]: 866

EM-86 2009 (22.100) Rf (3,6.000)

3.91e6

R:866 WILEY 75073: CITRONELLYL PROPIONATE ~~\$\$\$~~ 6-OCTEN-1-OL, 3,7-DIMETHYL-, PROPANOATE (CAS)

Hit 1

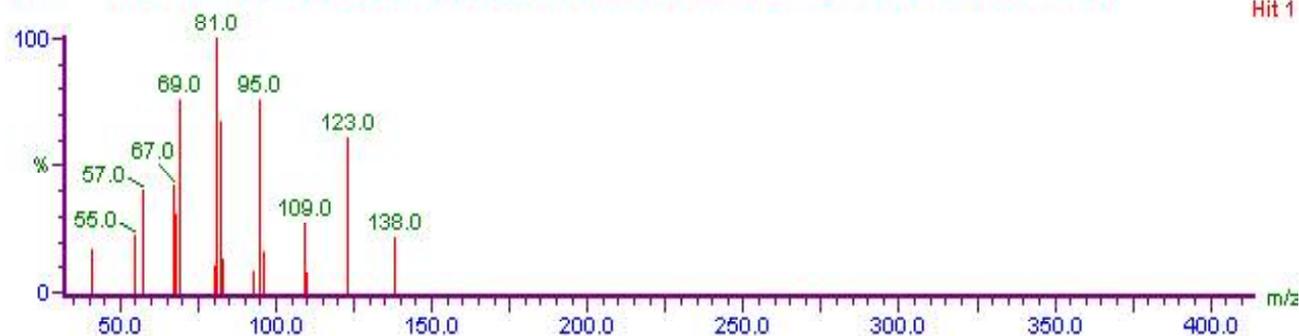


Fig. 13. Mass spectra of the methanolic extract component XIII of *Acalypha fruticosa* leaves of Chennai population.

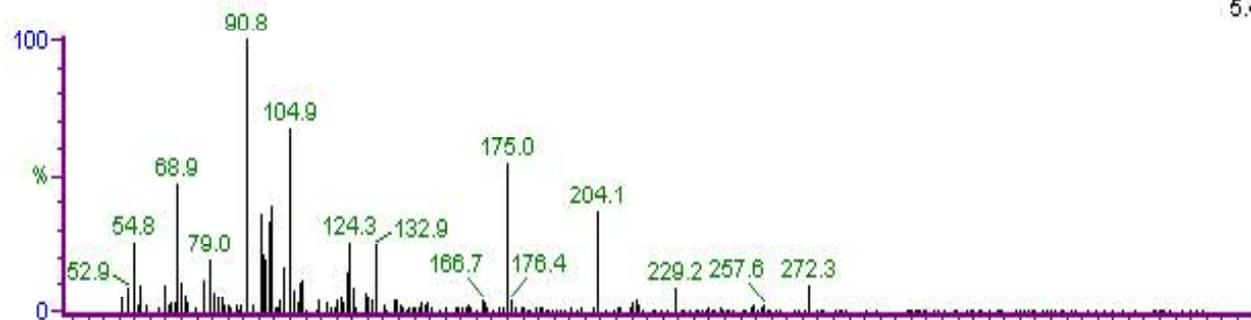
Sample ID: AND

Acquired on 18-Jul-2009 at 18:57:36

Reverse fit factor [REV]: 637

EM-86 2111 (23.121) Rf (3,6.000)

5.40e5

R:637 WILEY 55608: PIPERIDINE, 1-(2-PHENYLETHYL)- (CAS) ~~\$\$\$~~ N-BENZYL METHYLPIPERIDINE ~~\$\$\$~~ PIPERIDINE, 1-PHENETH'

Hit 8

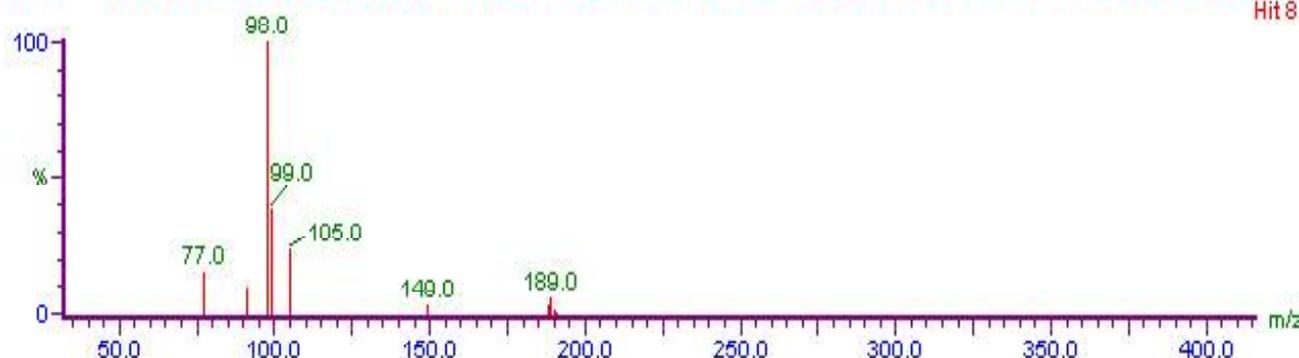


Fig. 14. Mass spectra of the methanolic extract component XIV of *Acalypha fruticosa* leaves of Chennai population.

Sample ID: AND

Acquired on 25-Sep-2009 at 12:37:05

Reverse fit factor [REV]: 51C

EM-225 2325 (25.263) Rt (3,6.000)

1.78e4

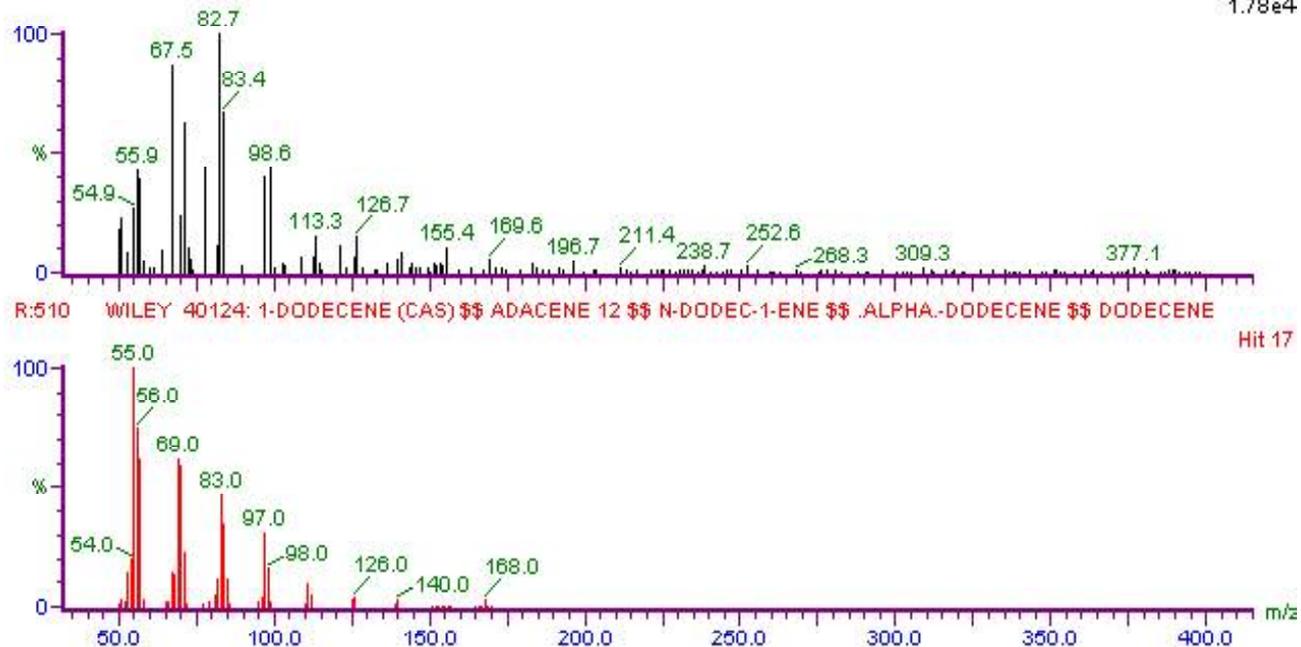


Fig. 15. Mass spectra of the methanolic extract component XV of *Acalypha fruticosa* leaves of Chennimalai population.

Sample ID: AND

Acquired on 18-Jul-2009 at 18:57:35

EM-86

Scan EI+

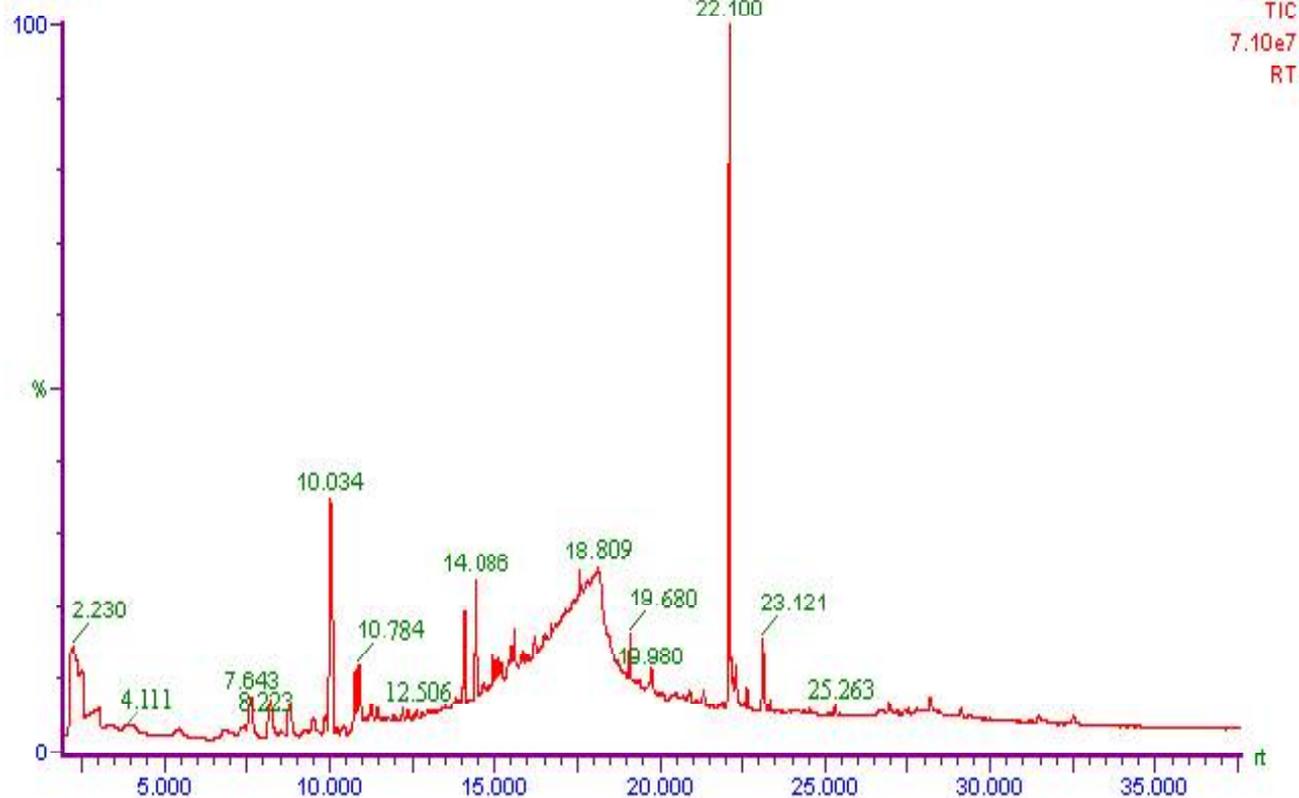


Fig. 16. Gas chromatogram of the methanolic extract of *Acalypha fruticosa* leaves of Chennimalai population.

diterpenoids, 1-Dodecene which has been reported to have many medicinal properties (Alma *et al.*, 2003). (Lei *et al.*, 2008) reported that this Dodecene diterpenoid natural compound have served as anti-tumor promoting agents. Generally terpenoids are an important volatile part of plants and play vital role in traditional herbal remedies. They are used as antibacterial, antineoplastic, anti-carcinogenic, antimalarial, anti-ulcer and hepaticidal and diuretic and other pharmaceutical functions (Rodriguez, 2004; Berteza, *et al.*, 2005).

CONCLUSION

The GC-MS identification studies may give a platform for lightening the traditional knowledge in a perpetual manner. Here we reported the presence of 15 different compounds in methanolic leaf part of *A. fruticosa* for exploring the drug leads in pharmaindustries. Thus, the GC-MS identified phytochemicals are indeed responsible for eliciting the traditional activity of this study species, *Acalypha fruticosa*. Futhermore, the phytochemical characterization of the extracts, the isolation of responsible bioactive compounds and their biological activity are necessary for future studies.

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