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Mass Spectrometric Analysis of Lipids Present in the Leaves of *Ailanthus excelsa*

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Abstract: Ailanthus excelsa leaves were analyzed for lipid contents. The dried leaves were subjected to soxhlet extraction using mixture of methanol and chloroform. The extracted fractions were separated by preparative Thin Layer Chromatography. The spots were scraped and dissolved in solvent, filtered and injected to Mass Spectrometer. Three lipids were identified by this method.
Keywords: *Ailanthus excelsa,* Lipids, Mass analysis**,** Soxhlet extraction.

Introduction

Recently, in India several scientists have reported the therapeutic importance of the chemical constituents of plants used in ancient Indian medical system. Mutalic [1] paper on Research Needs and Traditional Medicine in South East Asia Region has emphasized for research in traditional medicine. *Ailanthus excelsa* of the Acanthaceae family has been used in cough, asthma , bronchitis, tuberculosis, inflammation and allergy [2-5]. Several active constituents have also been isolated from different parts of *Ailanthus excelsa* [6]. Though the plant is used in the treatment of jaundice in Bengal, more evidence is needed to substantiate its pharmacological effects. From preliminary phytochemical analysis it was found that the extract showed positive response for the presence of flavanoids, tannins, alkaloids, reducing sugars and saponins [7].

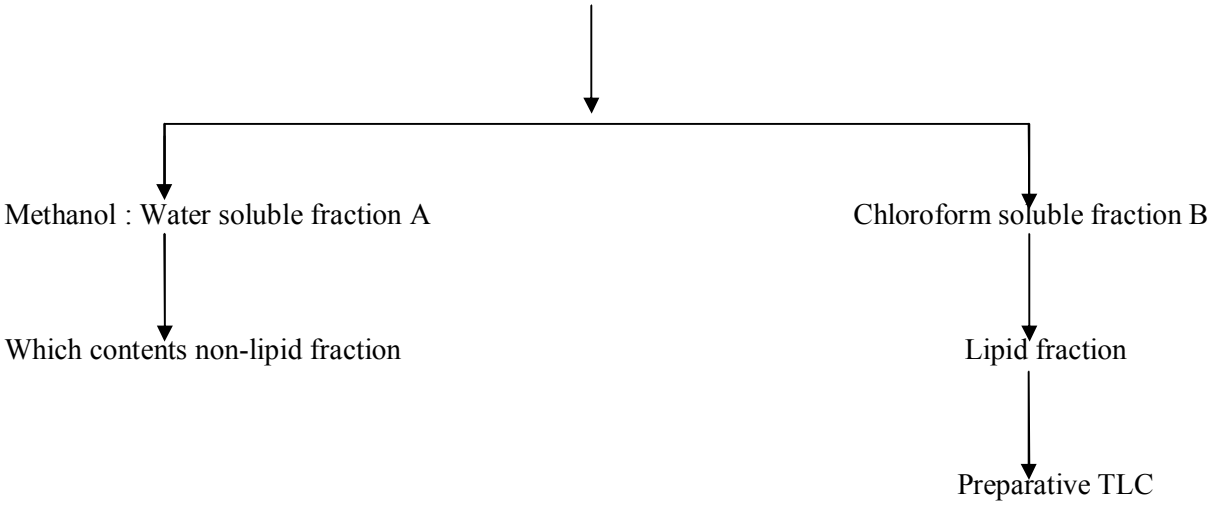
The term lipid is define as those substances which are insoluble in the organic solvents and soluble in includes wide range of compounds such as long chain hydrocarbons, alcohol, aldehydes, fatty acids, sterols, glycerides, wax, ester etc . which are found to be widely distributed in the plant and animal kingdom. The lipid can be separated in certain classes or groups, such as neutral lipids, sulfolipids, gylcolipids and phospholipids etc.[8]

Isolation of Lipids

The classical method of extraction and isolation of lipids have been described by Hilditch [9] and Kaufmann [10] , Games and Morries [11] have described a method for the extraction of polar lipids. The most popular method for initial isolation of lipids was described by Folch et al. [12] in which a chloroform : methanol (2:1) mixture of solvent to material in the ration of 20:1 used. One of the most versatile and effective lipid extraction process which largely overcomes all the difficulties like enzymatic degradation is that reported by Blog and Dyer [13].
It has been reported that this method gives excellent recovery of neutral lipids. More polar gylcolipids, sulphalipids and phosphorlipids are removed during back washing. Benzene : Methanol or toluene : ethanol have also been used to replace chloroform : methanol: water system. According to Kates, [14] the degradative enzymes are removed by treatment with hot isopropanol.

Separation of Lipids

Powdered dried level of Ailanthus excels- exracted with 2:1 chloroform : methanol mixture over a period of 24 hours.The extracts washed twice with 0.9%NaCl solution.



Chromatographic separation of lipid compounds

Crude lipid extracts by any of the above described method usually contains some amount of non lipid components it is necessary to removes them as completely as possible.One method is to wash chloroform : methanol extracts with 0.2 volume of water 0.9% sodium chloride solution.Another method is pass it through a column of sephadex G-25 or dextrant gel column chromatography has also been used by casey [15] for the sepration of neutral lipids of shark liver.

The selection of different solvents for elution of particular type of lipis has discussed by Rouser, [16] who has proposed a classification of chromatographic solvents based on their ability to act as proton donor for acceptors in hydrogen bonding or as hydrogen ion donor or acceptors.

In the present study a combination of several method has been followed for the sepration of saponifible and non saponifible principle.Initially the sepration of sepration of lipid components was carried out as per the scheme suggested by carroll [17] and modified by hardy et al [18] Sufficient care was taken to prevent degradation of the components by keeping the sample in vaccum desiccators.

Isolation of total lipid was also carried out by employing an altertative method of extraction with chloroform and the components were seprated by passing the fatty material through silica gel column.

All the lipid fraction except lipid fraction B were spotted on a TLC plate of silica gel and the chromatogram was developed using petroleum ether : ether : acetic acid (9:1:0.1) as solvent system. The detection of lipid has been described by mangold, [19] whit iodine vapors,2,7-dichloro fluorescing and 5% sulphuric acid in ethanol the R_f values obtain with the different detecting agent are recorded.

In the present study the plant material was extracted with petroleum ether(60-80)for the isolation of the total lipids. The analytical constant of the total lipid fraction i.e. acid value, saponification value were determined by the method described in British pharmacopeia. [20]

Experimental

75 g of the powdered leaves of *Ailanthus excelsa* were soaked in 1.5 liter of chloroform : methanol (2:1) mixture with intermittent shaking. The extracted material was filtered through whatman filter paper No. 1 and the marc was again soaked in 1.5 liter of chloroform : methanol(2:1) for further 12 hours with intermittent shaking and filtered. The filtrates were mixed and washed twice with 200ml portions of 0.9% sodium chloride solution in water to remove non-lipid components.

The chloroform soluble fraction-A contain lipids, after concentration under reduced pressure was passed through anhydrous sodium sulphate and then slowly evaporated on a waterbath and dried under vaccum.

The methanol : water soluble fraction-B containing non-lipid components like aminoacids, sugar was treated separately.

Thin Layer Chromatography

This method is based on partition phenomenon. Thin layer chromatography is at present an important analytical tool for quantitative and semiquantitative analysis of a number of natural products. The adsorbent such as silica gel G, alumina etc. is coated to a thickness of 0.2 mm on clean TLC plates using commercial spreader. The plates are activated at 105^{o}C for 30 minutes and used. The selection of mobile phase depends upon type of constituents to be analyzed. After the development of chromatogram by ascending technique, the resolved

spots are revealed by spraying with suitable detecting agent.

TLC has certain advantages, Separation can be effected more rapidly with smaller quantities of mixtures. This technique can be used in the analysis of almost all bio-constitutions The Rf value may vary depending upon purity if solvent, nature of substance to be resolved, composition of solvent, presence of impurities, adsorbent used etc.

TLC technique, now a days are important analytical tools for micro analytical separation in determination of purity of natural products. They have following advantages.

1. It is simple, economical and rapid method
2. Plates always available for use.
3. The method of detection on choice eluent, easily inspected.
4. Neutral, basic, acidic or purely aqueous solvents can be employed. The whole chromatography system if flexible.
5. TLC can be exploited in the investigation and evaluation of medicinal plants. It is possible to run many samples of extracted from different chemical races simultaneously with authentic standard and high performance individuable for discovery of so called chemical race. TLC study of the samples was carried out by using different conditions to evolve different chromatographic patterns.

Result and discussion

The lipid portion of the chloroform extract form leaves of *Ailanthus excelsa* has been determine by GC-MS analysis of the methyl ester mixture, three lipids were identifie.

100 g of lipid was methylated with MeOH 14% and the methyl ester obtained after work-up was analyzed by MS allowing the identification of 1,2-Benzenedicarboxylic acid, bis(2-ethylhexylester(CAS) Bis (2-ethylhexyl)phthalate($C_{24}H3_8O_4$), Bicyclo [2,2,1] Heptane-5-(ethyl-1-amine) ($C_9H_{17}N$) and 1,2-Benzenedicarboxylic acid diisotyl ester($C_{24}H3_8O_4$).

TLC Photograph of lipid of the leaves of *Ailanthus excelsa*

Table 1 : R_f Values of different spots of TLC

Detecting agent	R_f values of spots			
	1	2	3	4
U.V. light	-	-	-	-
I_2 vapors	0.10	0.43	0.70	0.74
5% H_2SO_4	0.36	0.47	0.55	0.71

Sample Information

Analyzed by :PANKAJ KACHHADIA
Analyzed : 2/1/2006 11:45:07 AM
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Sample ID : Ardusi-fraction-1 CHCl3 ext.

Similarity Search Result

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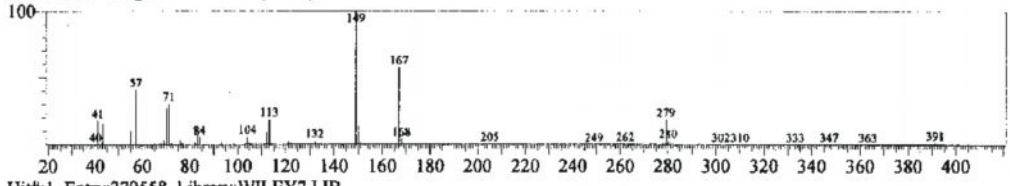

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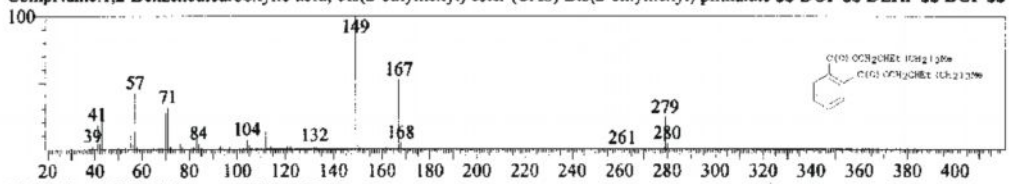

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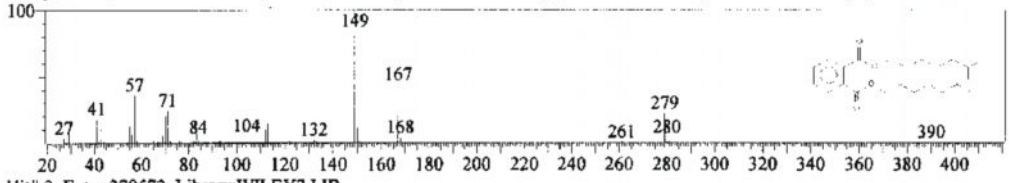

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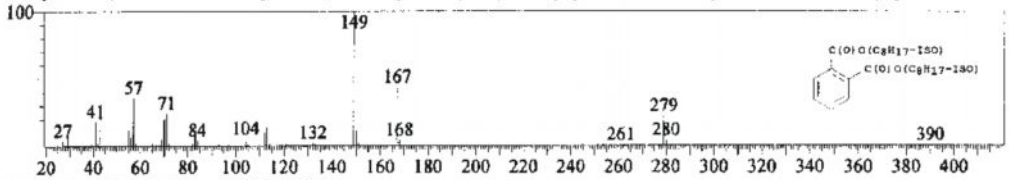

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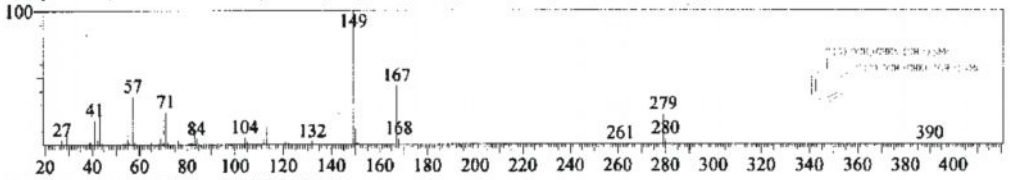

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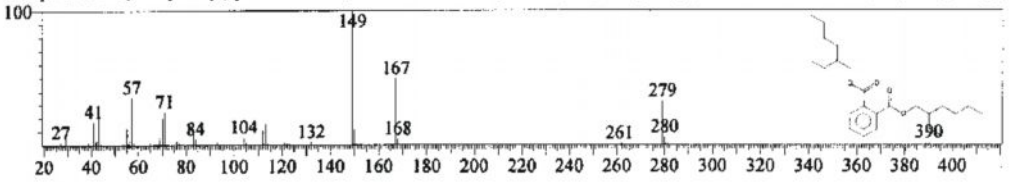

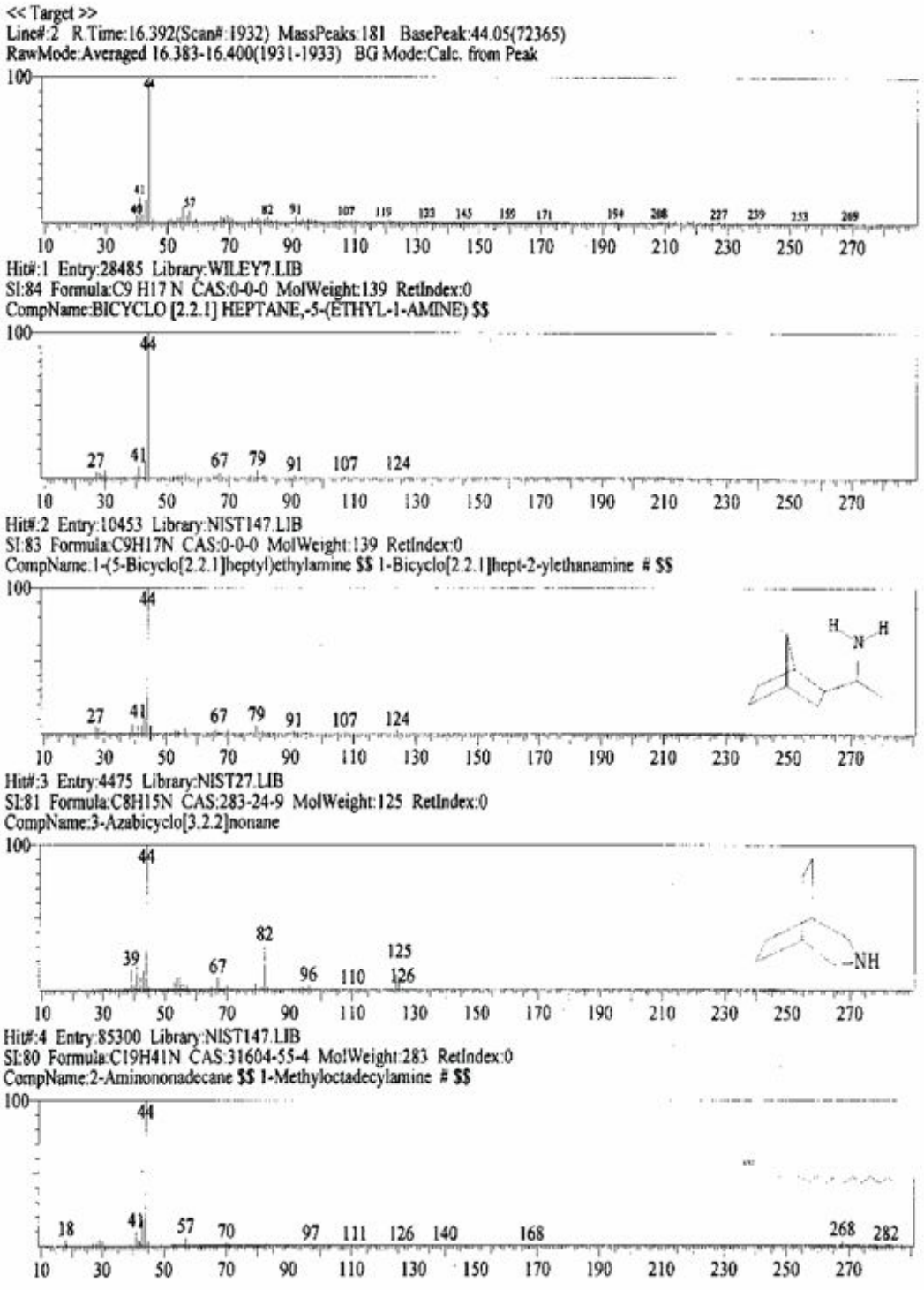
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CompName:BICYCLO [2.2.1] HEPTANE,-5-(ETHYL-1-AMINE) $$
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SI:83 Formula:C9H17N CAS:0-0-0 MolWeight:139 RetIndex:0
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Hit#:3 Entry:4475 Library:NIST27.LIB
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Hit#:4 Entry:85300 Library:NIST147.LIB
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Sample Information

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Analyzed : 2/1/2006 2:09:30 PM
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Sample ID : Ardusi-fraction-3CHCl3 ext.

Similarity Search Result

<< Target >>
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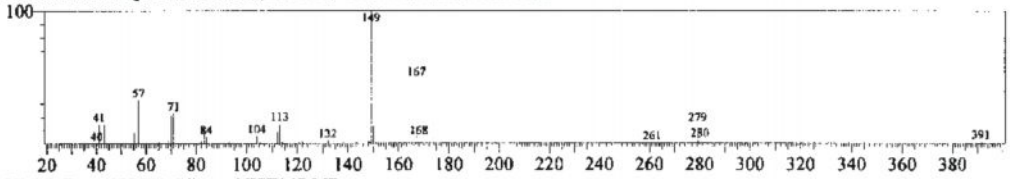

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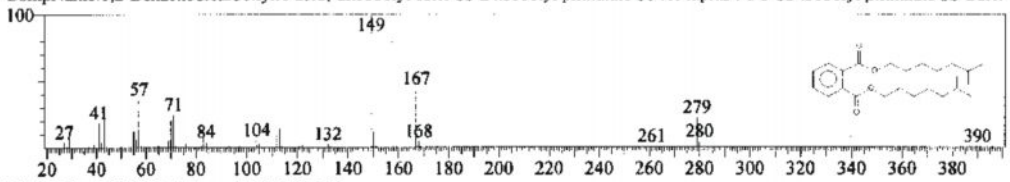

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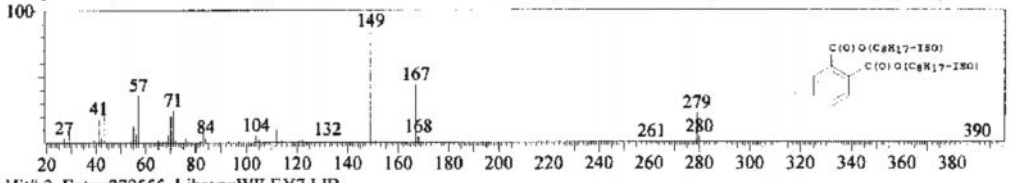
CompName:1,2-Benzenedicarboxylic acid, diisooctyl ester (CAS) Isooctyl phthalate $$ Hexaplas M/O $$ Diisooctyl phthalate $$ D



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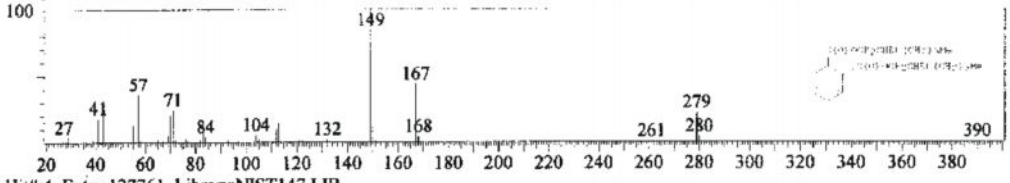

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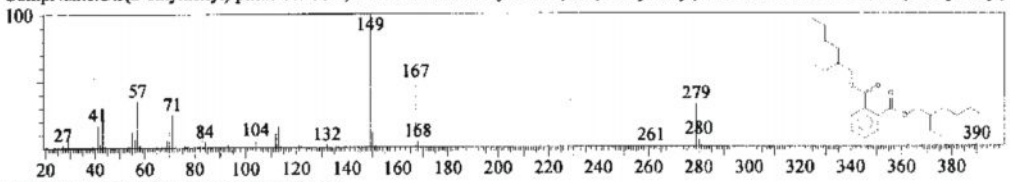

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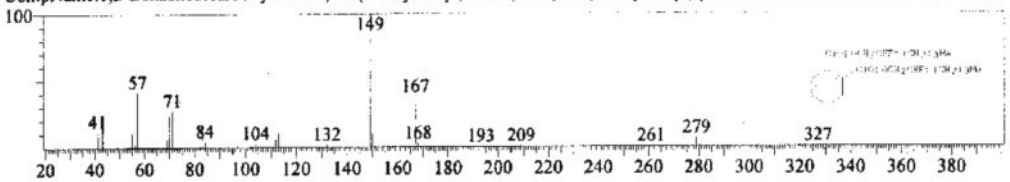

References :

1. Mutalik G.S., Background paper no.SEA/RPD/ Tradmed/RSG Meet, WHO office, New Delhi, 1/2, 1978.
2. Dhueley J.N., Antitussive effect of Ailanthus Excelsa extract on mechanical of chemical stimulation – induced coughing in animals, J. Ethnopharmacol 1999, 67, 361-365.
3. Grange J.M. and Snell N.J., Activity of bromhexine and ambroxol, semi-synthetic derivatives of vasicine from the Indian shrub Audhatoda vasica, against Mycobacterium tuberculosis in vitro. J. Ethnopharmacol 1996, 510, 49-53.
4. Chakraboty, A. and Brantner A.H, Study of alkaloids Audhatoda Vasica Nees on their anti-inflammatory activity, Phytother Res 2001, 15, 532-534.
5. Wagner H. Search for new plants constituents with potential antiphlogistic and antiallergic activity, Plant Med.,1989, 55, 235-241.
6. Recknagel R.O., Carbon tetrachloride hepato - toxicity. Pharmacol Rev., 1967, 19, 145-208.
7. Bhattacharya D..Mukherjee R., Pandit S., Das N. and Sur T.K., Prevention if carbon tetrachloride induced hepatotoxic in rats by Himoliv, a poluherbal formulation. Indian J pharmacol, 2003, 35, 183-185.
8. Denis J.M., Plant Lipids: Biology, Utilisation and manipulation, 2005.
9. Hildith T. P. and Willia P. N., The chemical constitution of Natural Fats, 4th ed. John Willey and Sons. Inc. N.Y. 1964.
10. Kaufman H. P. cited by Sthal E., thin layer chromatography Spinger verlag,berlin-1964, 368.
11. James A.T. and Morris L. J., New Biochemical seperations, London, Van Nostrand, 1964, 321.
12. Folck J.,Less M.and Slone S.G.H.,J.bio chem., 1957, 226, 497
13. G. H. Bligh and W.J.Dyer chand, J.Bio chem., 1957,226,497.
14. Kates M., Advan. Lipid Reaserch,1970,8,226.
15. Casey A.C., J.Pipid Res.,1969,10,456.
16. Rouser G., Chromato. J., Sc.,1973,11,60.
17. Carrol K.K. and Lipid J.,Res.,1961,2,135.
18. Hardy R.,Smith J.and Making P.R.,J. Chromato, 1971, 57,142
19. Mangold J.K., cited by Sthal. E. Thin Layer chromatography, Spinger verlay, berlin, 1969, 363.
20. British Phamacopoeia (Annonymous) London 1973.

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