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# GC-MS studies of the fatty acids obtained from leave extract of ailanthus excelsa

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## ABSTRACT

The dried powdered leaves of *Ailanthus excelsa* was soaked over night in petroleum ether and continuously extracted for several hours in soxhlet extraction apparatus followed by reduced pressure distillation of petroleum ether to obtain the concentrated extract. The extract was then subjected to alkaline hydrolysis and the saponifiable fraction is acidified and extracted with benzene. The organic fraction containing fatty acid is subjected to esterification to obtain methyl esters. The major components of the extract were identified using gas chromatography/mass spectrometry (GC-MS). 

## 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. A pioneering work by Kirtikar and Basu[2], Chopra et al.[3] And Nadkarni[4] have led to compilation of information with regard to occurrence, identity and therapeutic properties and chemical constituents of such plants remedies particularly used in Ayurvedic and Unani system of medicine. It may be wordwhile to note that considerable progress towards the isolation and characterization of active constituents from such plants have been worked out during last thirty years with the help of newer scientific techniques like Thin layer chromatography (TLC),Gas liquid chromatography (GLC), High performance chromatography (HPLC), Fourier transform infrared spestroscopy (FT-IR), Nuclear magnetic resonance spectroscopy (NMR), Mass spectrometry (MS), gas chromatography/mass spectrometry (GC-MS) and other instrumental techniques[5]. *Adhatoda vasica Nees (AV)* of the *Acanthaceae* family has been used for thousands of years in India. Extracts of the leaves of AV are extensively used in cough, asthma, bronchitis, tuberculosis, inflammation and allergy[6-9]. Several active constituents have also been isolated from different parts of AV[10]. Though the plant is traditionally used in the treatment of jaundice in Bengal, and 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 flavonoids, tannins, alkaloids, reducing sugars and saponins[11].

Fatty acids are an important group of related organic acids. Many of which occurs in nature, the higher member being combined with glycerol in the form of esters known as triglycerides. Various mixtures of triglycerides consists the neutral fats and oils. In the plants there are fewer individual fatty acids but their can be a great diversity of types. In addition to the strait chain saturated fatty acids and unsaturated fatty acids of the

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types out line above, a wide variety of fatty acids occur which may have ethylenic double bond in usual position or trans conjugated acetylenic and other types of unsaturated singly in combination or occur with hydroxyl or lacto group.

Fatty acids are long linear hydrocarbon chains containing from 4 to 30 hydrocarbons, most commonly 12 to 24 carbons. One end of the molecule contains a carboxylic acid group from which chemists observed the number of carbons. The other end is the methyl, "n" or omega end from which nutritionists and biochemists observed the position of the first double bond. Location of the first double bond end determines whether the fatty acid is an omega-6 or omega-3 fatty acid.

Fatty acids having their full complement of hydrogen atoms are termed saturated. These exist as straight chains. The most abundant saturated fatty acid in nature is palmitic acid, a 16-carbon fatty acid. If two hydrogen atoms are removed from the chain, a carbon-to-carbon double bond or point of unsaturation is created and the molecule bends.

This bending causes the molecule to occupy more space and become more fluid. Fatty acids with one double bond are called mono unsaturated. The most common mono unsaturated fatty acid is oleic acid, an 18-carbon fatty acid with its double bond 9 carbons from the methyl end. Oleic acid is the major fatty acid in olive oil. Polyunsaturated fatty acids have two or more double bonds and are most common in the omega-6 and omega-3 structures. For example, linoleic acid (LA), is an 18-carbon omega-6 fatty acid, with two double bonds. It is abundant in plants and seed oils. Its counterpart in the omega-3 class is alpha-linolenic acid (ALA), an 18-carbon fatty acid with three double bonds, also found in plant lipids and oils.

### Isolation of fatty acids

Fatty acid has been isolated from the dried powder of leaves of Ailanthus excelsa by continuously extracted with petroleum ether and subsequently subjected the extracted material to alkaline hydrolysis. The petroleum ester extracted was saponified as per the method described by E.stahl[12]. Using methanol potassium hydroxide. The combined fatty acid presents in the extract after saponification yield the water soluble alkali salts of fatty acids. The non-saponifiable matter is removed by extraction with non polar solvents like ether benzene etc. The saponificable material was followed by liberations of mixed fatty acid from the soap by addition of mineral acids.

The saponifiable fraction was acidified with 5N $H_2SO_4$ to liberate fatty acids and then extracted with benzene was further purified by passing it through a small column of alumina and eluted with benzene. The fatty mixture after removal of benzene was preserved in a tightly closed container and stored at low temperature.

### Separation of fatty acids

It is generally difficult to separate higher fatty acids by simple chemical method. Usually they are converted into their methyl esters.

## RESULTS AND DISCUSSION

The fatty portion of the petroleum ether extract from leaves of Ailanthus excelsa has been determined by GC-MS analysis of the methyl ester mixture. Nine fatty acids were identified and methyl palmitate (29.80%) was the major component.

The clear viscous brownish extract obtained after petroleum ether extraction of ground roots of Ailanthus excelsa, yielded a precipitate after standing at room temperature. Silica gel chromatography column of the precipitate allowed the isolation of lipid and fatty acid. Course chromatography of the mother liquor yielded a non-polar fraction after elution with petroleum ether designated. Hydrolysis with KOH/EtOH (1:1) solution, yielded the free fatty acids mixture and a unsaponifiable portion after usual work up. Esterification of an aliquot of with methanol 14% solution followed by GC-MS analysis of the methyl ester mixture allowed the identification of the fatty acids listed in the experimental GC-MS analysis of this fraction, followed by comparision of the mass spectra obtained for each compound with a computer library data bank, and literature data. The FT-IR spectrum confirmed the ketone moiety (1723 $cm^{-1}$), its carbinolic character (3300and 1020 $cm^{-1}$) and the exocyclic double bond [3100 (=C-H), 1642 (C=C) and 900$cm^{-1}$ (=C-H)]. The EI-Mass spectrum showed the molecular ion [M]+ at m/z 74 compatible with the molecular formula $C_{17}H_{34}O_2$ corresponding to a struc-

ture if a carbonyl and a carbon-carbon bond have already been characterized acetylation of 1 yielded a colorless solid, designated 1a, [M]+ m/z 404 ($C_{24}H_{36}O_5$).

100 mg of the fatty acids were methylated with methanol 14% and the methyl ester mixture obtained after work-up was analyzed by GC-MS allowing the identification of methyl myristate (C17:0, 29.80%), Methyl margarate (C18:0,1.55%), 2-Hexadecenoic acid, methylester (C17:0, 0.72%), Octadecanoic acid, methyl ester (C19:0, 15.45%), Methyl 9-octadecenoate (C19:0, 26.57%), Methyl linolate (C19:0, 4.94%), 8,11-Octadecadienoic acid, methyl ester (C19:0, 4.87%), Methyl arachate (C21:0, 7.45%), 9,11-Octadecadienoic acid, methyl ester (C19:0, 8.66%).

## EXPERIMENTAL

### Isolation of fatty acid

The dried powdered leaves (75gm) of Ailanthus exelsa was soaked over night in petroleum ether and continuously extracted for 8 hours in soxhlet extraction apparatus. Petroleum ether was distilled off under reduced pressure and concentrated extract was obtained.

### Alkaline hydrolysis

20gm of KOH was dissolved in 20 ml of distilled water and after cooling this concentrated solution was diluted with 80ml of methanol. 10gm of the petroleum ether extract was mixed with 100 ml of methanolic KOH (20%) and kept overnight at room temperature (22-30$^0$C). Next day the mixture was refluxed under water condenser over a boiling water bath for 3-4 hours. After saponification bulk of methanol was evaporated off under reduced pressure below 50$^0$C and the aqueous phase was diluted with 200 ml of distilled water. The unsaponified material was taken out by extracting thrice with 100 ml portion of ether.

### Extraction of fatty acids

The diluted aqueous phase containing potassium hydroxide acidified with 5N $H_2SO_4$, under a layer of diethyl ether. The acidified aqueous layer was extracted thrice with 50 ml portion of diethyl ether. The ether layer containing fatty acid was washed over anhydrous sodium sulphate. The dried fatty acid mixture was properly preserved in a tightly closed container at low temperature.

### Methylation of fatty acids

One gm portion of the mixture of fatty acids was heated with 100 ml mixture of absolute methanol: benzene: con. $H_2SO_4$ (36:10:4) for eight hours at 80-90$^0$C over a water bath under a long steam air condenser. The reaction mixture was cooled and diluted with 75 ml of water. This was then extracted five times with 25 ml portion of hexane. The combined hexane extract containing methyl esters of fatty acids was dried over sodium bicarbonate: anhydrous sodium sulphate mixture (1:3). The dried mixture of esters of fatty acids was preserved in tightly closed container at low tempreture.

### Characterization of fatty acid esters by GC-MS

Instrument that is used for analysis is Shimadzu make GC-MS QP2010. Methyl esters of fatty acids were taken up in minimum quantity of chloroform and microlitre quantity injected into GC-MS. The composition of the volatile constituents was established by GC–MS analyses. GC–MS analyses were performed on a Shimadzu GCMS-QP2010 system in EI mode equipped with a split/split less injector (250$^0$C), at a split ratio of 1/10, using a SGE make BPX5 WCOT (Wall coated open tubular) capillary column (30m×0.25 mm i.d., ×0.25μm film thickness). The oven temperature was isothermally maintained at 100$^0$C. Helium was used as a carrier gas at a flow rate of 2.5ml/min. The injection volume of each sample was 2μl. The chromatogram has been reported in figure 1 and the identify due to comparision with library is maintained in the TABLE 1.

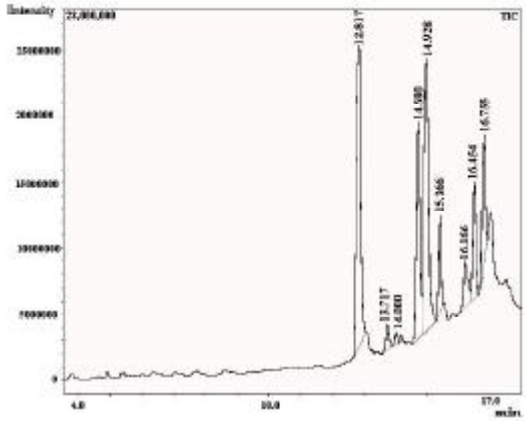

**Figure 1 : GC-MS spectrum of fatty acid esters mixture**

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**TABLE 1 : Table of separated fatty acids ester**

| No. | Name | R.Time | I.Time | F.Time | Area | Height | Area % |
|---|---|---|---|---|---|---|---|
| 1 | Methyl myristate | 12.817 | 12.650 | 13.058 | 229892649 | 23247107 | 29.80 |
| 2 | Methyl margarate | 13.717 | 13.642 | 13.883 | 11971034 | 1966692 | 1.55 |
| 3 | 2-hexadecanoic acid methyl ester | 14.000 | 13.908 | 14.092 | 5541516 | 1036176 | 0.72 |
| 4 | Octadecanoic acid methyl ester | 14.680 | 14.533 | 14.800 | 119175758 | 16458241 | 15.45 |
| 5 | Methyl, 19-ectadecanoate | 14.928 | 14.800 | 15.250 | 205022462 | 20868444 | 26.57 |
| 6 | Methyl linolate | 15.366 | 15.275 | 15.475 | 38120246 | 7466529 | 4.94 |
| 7 | 8, 11-Octadecadienoic acid, Methyl ester | 16.166 | 16.042 | 16.350 | 37580992 | 3509280 | 4.87 |
| 8 | Methyl arachate | 16.454 | 16.350 | 16.680 | 57469556 | 9006619 | 7.45 |
| 9 | 9, 11-Octadecadienoic acid, Methyl ester | 16.755 | 16.625 | 16.875 | 66785028 | 10057472 | 8.66 |

## CONCLUSION

The GC–MS analyses of the chemical constituents of the fatty acids from leaves of Ailanthus Excelsa, which were obtained after Soxhlet extraction, alkaline hydrolysis and esterification, are good tools for helping to identify the fatty acids.

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