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Research Article

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GC-MS analysis of bioactive constituents of *Indigofera suffruticosa* leaves

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ABSTRACT

To characterize the chemical constituents of plant of Indigofera suffruticosa leaves using GC-MS. The shade dried leaves powder was extracted with ethanol by using Soxhlet extractor. The Clarus 500 GC used in the analysis employed a column packed with Elite-1 (100% dimethyl poly siloxane, 30 nm × 0.25 nm ID × 1 µM df) and the components were separated using Helium (1 mL/min) as the carrier gas. The 2 µL sample extract injected into the instrument was detected by Turbo gold mass detector (Perkin Elmer) with the aid of the Turbo mass 5.1 software. The GC-MS analysis provided different peaks determining the presence of nine different phytochemical compounds namely pentadecanoic acid,14-methyl-,methyl ester (5.86%), n-hexanedecanoic acid (9.83%), z-[13, 14-epoxy]tetradec-11-en-1-ol acetate(6.37%), oleic acid(10.43%), 9-octadecenoic acid[z]-,2-hydroxy-1-[hydroxyl methyl]ethyl ester(10.21%), heptanoic acid, docosyl ester(6.28%), octadecanoic acid, 7-hydroxy-, methyl ester(4.89%), 6-octadecenoic acid[z]-(18.47%), and 8-octadecenoic acid, methyl ester(14.97%).The bioactive compounds in the ethanolic extract of Indigofera suffruticosa leaves have been screened using this analysis. Isolation of individual phytochemical constituents may proceed to find a novel drug.

Key words: Phytochemical constituents, Ethanol extract, GC-MS, n-Hexanedecanoic acid.

INTRODUCTION

Medicinal plants have occupied an important position in the socio-cultural, development of rural people of India. Plants and leaves are as considered one of the main sources of biologically active compounds. Even today compounds from plants continue to play a major role in primary health care as therapeutic remedies in many developing countries [1]. Plant-based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, *etc* [2]. Screening active compounds from plants has lead to the invention of new medicinal drugs which have different protection and treatment roles against various diseases, including cancer [3] and Alzheimer's disease [4]. The modern methods describing the identification and quantification of active constituents in plant material may be useful for proper standardization of herbal and its formulations. GC-MS is the best technique to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds etc [5].

Indigofera suffruticosa is an Indian herb used for various ailments by traditional healers. *Indigofera suffruticosa* is a Wild indigo, also known as indigo, Guatamala indigo, anil, anil de Pasto, and ti cafe, is a short-lived shrub that reaches 1 to 2 m in height and 1 to 2 cm in stem. *Indigofera Suffruticosa* species became important commercial crops in various tropical and subtropical areas. Poultices and extracts of wild *indigofera suffruticosa* leaves, alone or in combination with other ingredients, are used in herbal medicine to treat fever, headaches, hemorrhages,

convulsions, acute cough, skin parasites, and boils[6]. A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. With this background the present study was aimed to identify the phytoconstituents in *indigofera suffruticosa* leaves by using GC-MS analysis.

EXPERIMENTAL SECTION

2.1 Collection and preparation plant material

The fresh plants *indigofera suffruticosa* leaves were collected from Vaithiyanathapuram Village in Perambalur district of Tamil Nadu, India during January to December 2012 and authenticated by director of the Rapinat Herbarium and Centre for Molecular Systematic, St. Joseph's college (campus), Trichirappalli, Tamil Nadu, and India. The sample were washed thoroughly in running up tap water to remove soil particles and adhered debris and finally washed with sterile distilled water. The whole plants were shade dried and ground into fine powder. The powdered materials were preserved in airtight containers until use.

2.2 Extraction procedure

The powdered sample of *indigofera suffruticosa* leaves (100g) were extracted with ethanol (500ml, 46 h) at temperature between 55-60^{0}C by using Soxhlet extractor. The solvent was evaporated by rotavapor (Yamato Rotary Evaporator, Model RE-801) to obtained viscous semi solid masses. The semi dry ethanol crude extract was suspended in water and it analyzed by GC-MS, it had led to the identification and characterization of nine different organic compounds, representing 4.09% of the total extract from plant samples. The crude extracts were filtered separately through Whatman No. 41 filter paper to obtained dust free plant crude extract. The residue was re-extracted twice follow the same and filtered. The combined extracts were concentrated and dried by using rotary evaporator under vacuum.

2.3 GC-MS analysis

The Clarus 500 GC used in the analysis employed a fused silica column packed with Elite-1 (100% dimethyl poly siloxane, 30 nm × 0.25 nm ID × 1µm df) and the components were separated using Helium as carrier gas at a constant flow of 1 mL/min. The µL sample extract injected into the instrument was detected by the Turbo gold mass detector (Perkin Elmer) with the aid of the Turbo mass 5.1 software. During the 36th minute GC extraction process, the oven was maintained at a temperature of 110^{0}C with 2 minutes holding. The injector temperature was set at 250^{0}C (mass analyzer).

The different parameters involved in the operation of the Clarus 500 MS, were also standardized (Inlet line temperature: 200^{0}C; Source temperature: 200^{0}C). Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 450 Da.

2.4 Identification of components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology having more than 62,000 patterns. The spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS

The results pertaining to GC-MS analysis led to the identification of number of compounds from the GC fractionations of the ethanolic extract of *indigofera suffruticosa* leaves. These compounds ware identified through mass spectrometry attached with GC. The results of the present study were tabulated in Table 1. The compound prediction is based on National Institute Standard and Technology Database. The results revealed that the presence of pentadecanoic acid,14-methyl-,methyl ester (5.86%), n-hexanedecanoic acid (9.83%), z-[13, 14-epoxy]tetradec-11-en-1-ol acetate(6.37%), oleic acid(10.43%), 9-octadecenoic acid[z]-,2-hydroxy-1-[hydroxyl methyl]ethyl ester(10.21%), heptanoic acid, docosyl ester(6.28%), octadecanoic acid, 7-hydroxy-, methyl ester(4.89%), 6-octadecenoic acid[z]-(18.47%), and 8-octadecenoic acid, methyl ester(14.97%). The spectrum profile of GC-MS confirmed the presence of nine major components with the retention time 16.2, 17.07, 18.1, 19.38, 20.98, 21.5, 23.78, 18.75, and 17.95 respectively (Figure: 1).The individual fragmentation of the components were illustrated in (Figure 2A-2I).

Table 1: Components detected in the plant of ethanol extract of *indigofera suffruticosa* leaves

S.No	Compound Name	Molecular Formula	MW	RT	Peak Area	%Peak Area
1.	pentadecanoic acid,14-methyl-,methyl ester	$C_{19}H_{34}O_2$	270	16.2	20540448	5.86
2.	n-hexanedecanoic acid	$C_6H_{22}O_2$	256	17.07	34426640	9.83
3.	z-[13, 14-epoxy]tetradec-11-en-1-ol acetate	$C_{16}H_{28}O_3$	268	18.1	22312656	6.37
4.	oleic acid	$C_{18}H_{34}O_2$	282	19.38	36524672	10.43
5.	9-octadecenoic acid[z]-,2-hydroxy-1-[hydroxyl methyl]ethyl ester	$C_{21}H_{40}O_4$	356	20.98	35779824	10.21
6.	heptanoic acid, docosyl ester	$C_{29}H_{58}O_2$	438	21.5	22003648	6.28
7.	octadecanoic acid, 7-hydroxy-, methyl ester	$C_{19}H_{38}O_4$	330	23.78	17125360	4.89
8.	6-octadecenoic acid[z]-	$C_{18}H_{34}O_2$	282	18.73	67678496	18.47
9.	8-octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	17.95	52428240	14.97

MW: Molecular Weight, RT: Retention Time

Table 2: Activity of Phyto-Components identified in the ethanol extracts of the plant of *Indigofera suffruticosa* Leaves

S.No	Name of the compound	Molecular formula	Nature of compound	**Activity
1.	Pentadecanoic acid,14-methyl-,methyl ester	$C_{19}H_{34}O_2$	Palmitic acid methyl ester	Antioxidant.
2.	n-hexanedecanoic acid	$C_6H_{22}O_2$	Palmitic acid	Antioxidant, Hypochloesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Haemolytic, 5-Alpha reductase inhibitor.
3.	z-[13, 14-epoxy]tetradec-11-en-1-ol acetate	$C_{16}H_{28}O_3$	Triterpenic acid	Antioxidant, Haemolytic.
4.	oleic acid	$C_{18}H_{34}O_2$	Fatty acid	Cancer preventive, Anemiagenic, Insectifuge, Antiandrogenic, Dermatitigenic.
5.	9-octadecenoic acid[z]-,2-hydroxy-1-[hydroxyl methyl]ethyl ester	$C_{21}H_{40}O_4$	Fatty acid ethyl ester	No activity reported.
6.	heptanoic acid, docosyl ester	$C_{29}H_{58}O_2$	-	No activity reported.
7.	octadecanoic acid, 7-hydroxy-, methyl ester	$C_{19}H_{38}O_4$	Fatty acid ester	Antioxidant
8.	6-octadecenoic acid[z]-	$C_{18}H_{34}O_2$	Stearic acid	Cancer preventive, Insectifuge.
9.	8-octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	Fatty acid ester	Antioxidant, Antimicrobial.

***Activity source: Dr. Duke's Phytochemical and Ethnobotanical Database*

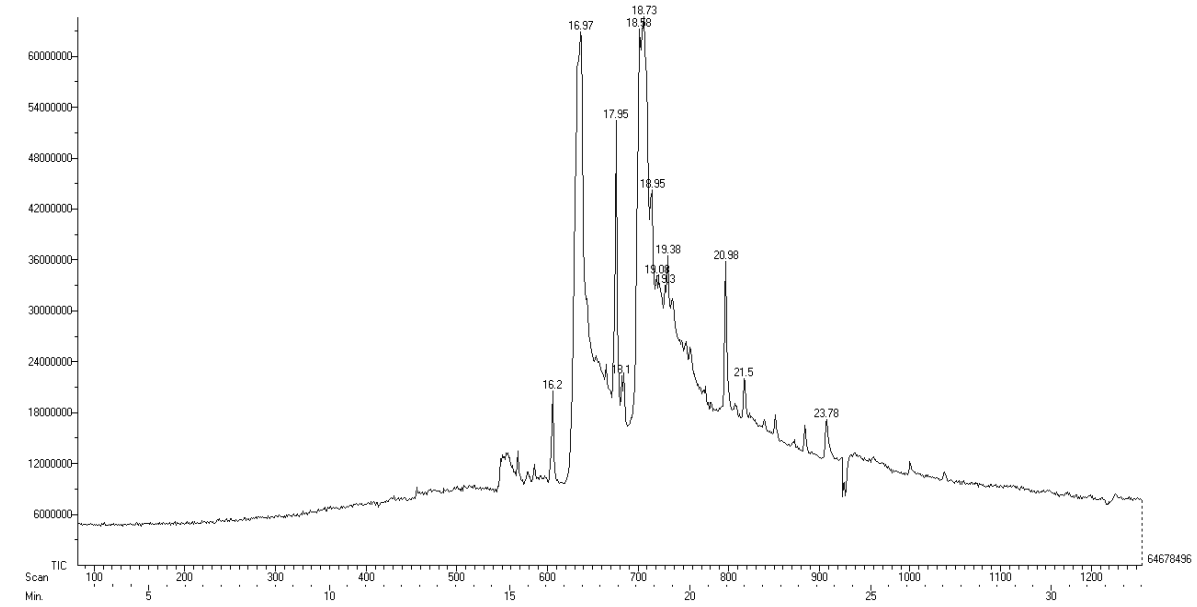

Figure: 1 GC-MS Chromatogram of ethanolic extract of the leaves of *Indigofera Suffruticosa*

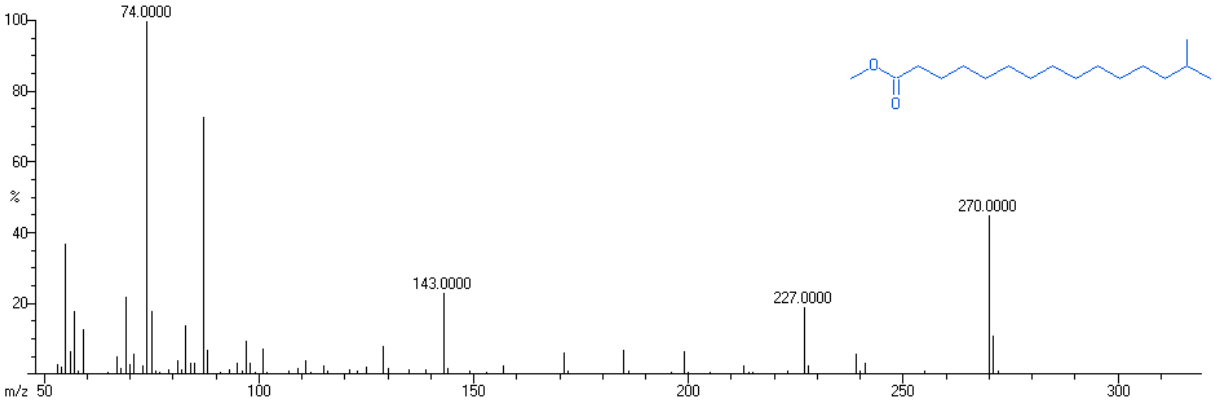

Figure: 2A Mass spectrum of pentadecanoic acid, 14-methyl-,methyl ester. (RT: 16.2)

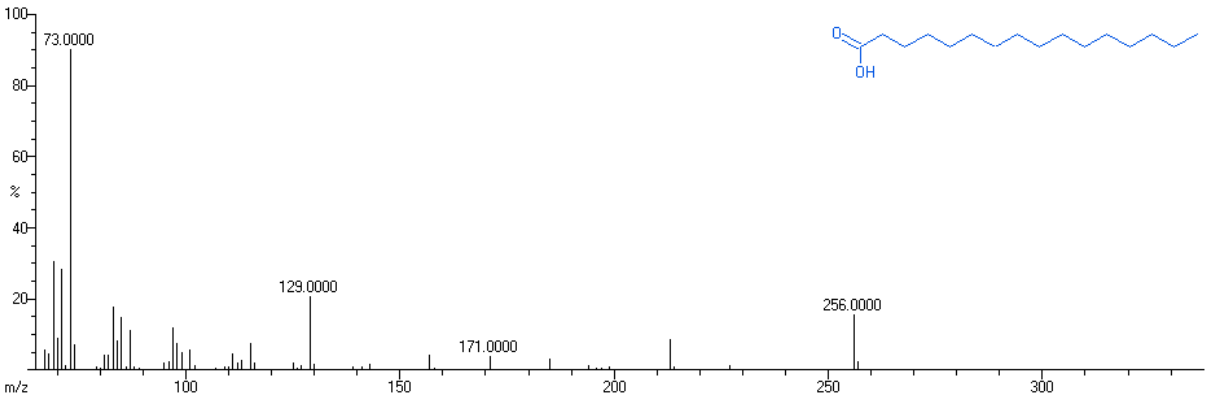

Figure: 2B Mass spectrum of n-hexadecanoic acid. (RT: 17.07)

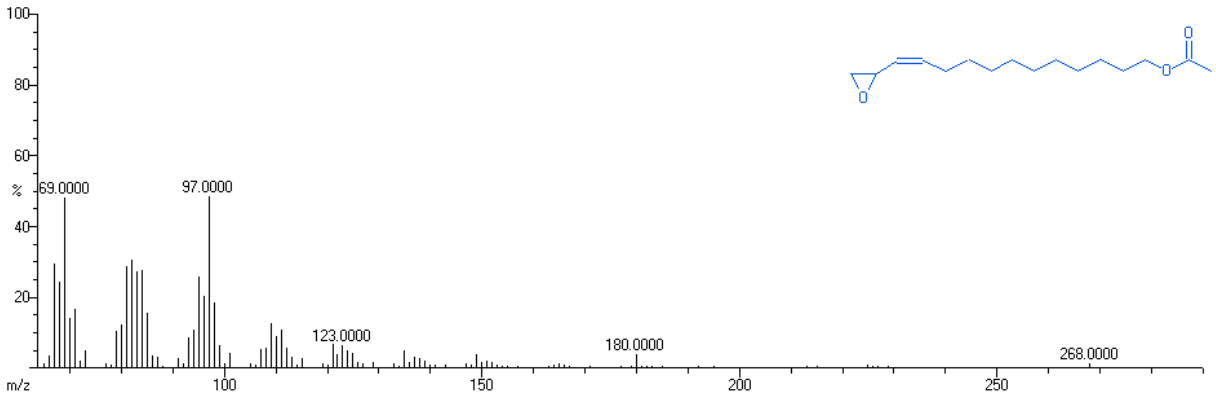

Figure: 2C Mass spectrum of z-[13, 14-epoxy] tetradec-11-en-1-ol acetate. (RT: 18.1)

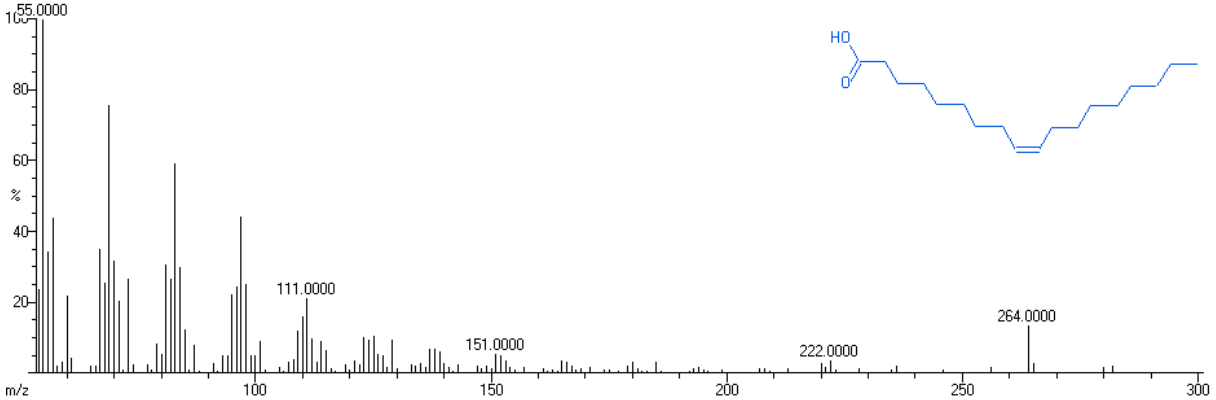

Figure: 2D Mass spectrum of oleic acid. (RT: 19.38)

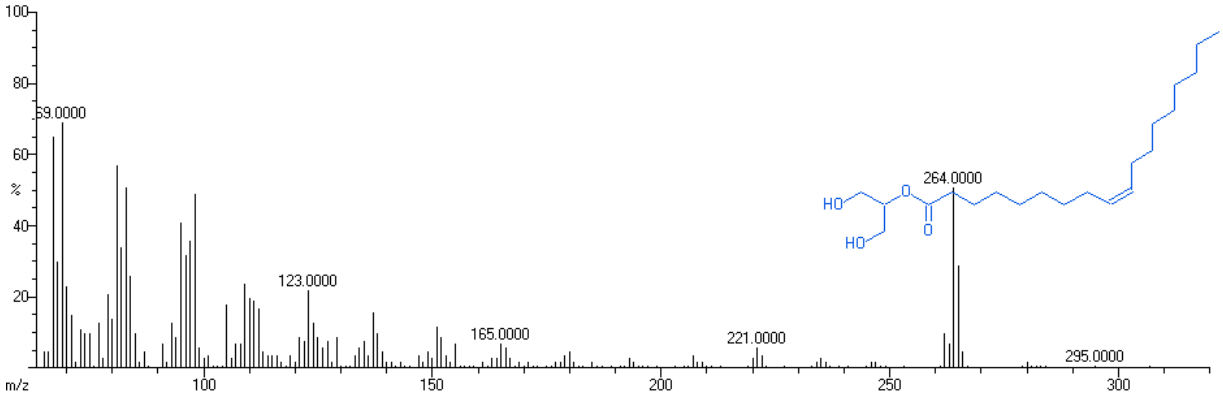

Figure: 2E Mass spectrum of 9-octadecenoic acid[z]-, 2-hydroxy-1-[hydroxyl methyl] ethyl ester. (RT: 20.98)

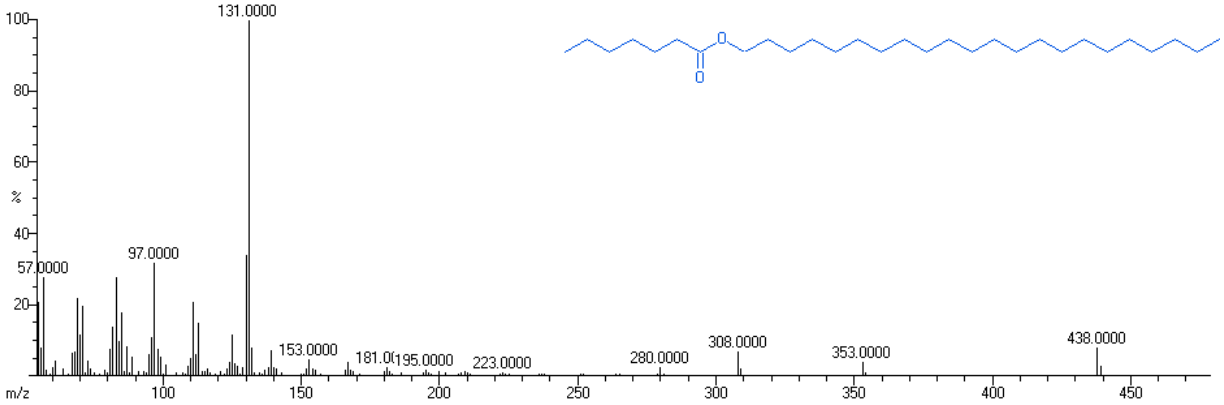

Figure: 2F Mass spectrum of heptanoic acid, docosyl ester. (RT: 21.5)

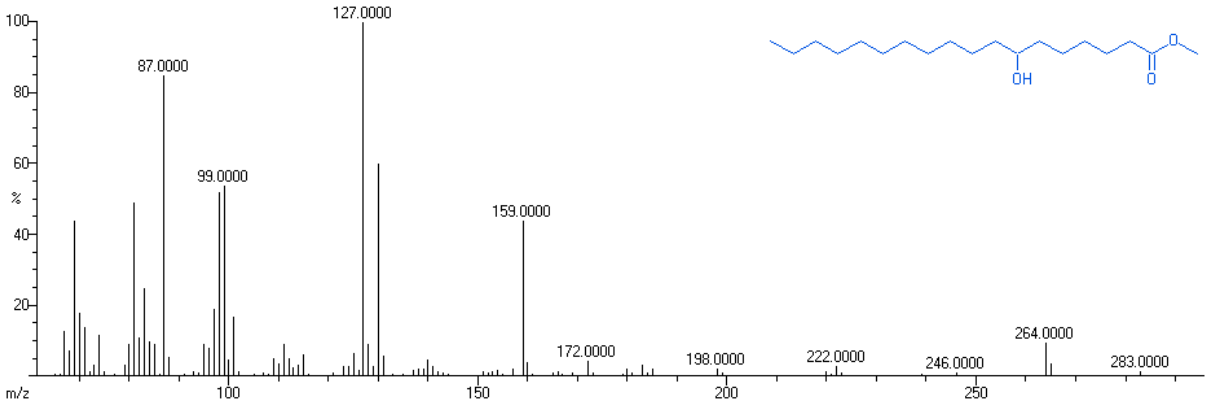

Figure: 2G Mass spectrum of 9-octadecanoic acid, 7-hydroxy-, methyl ester. (RT: 23.78)

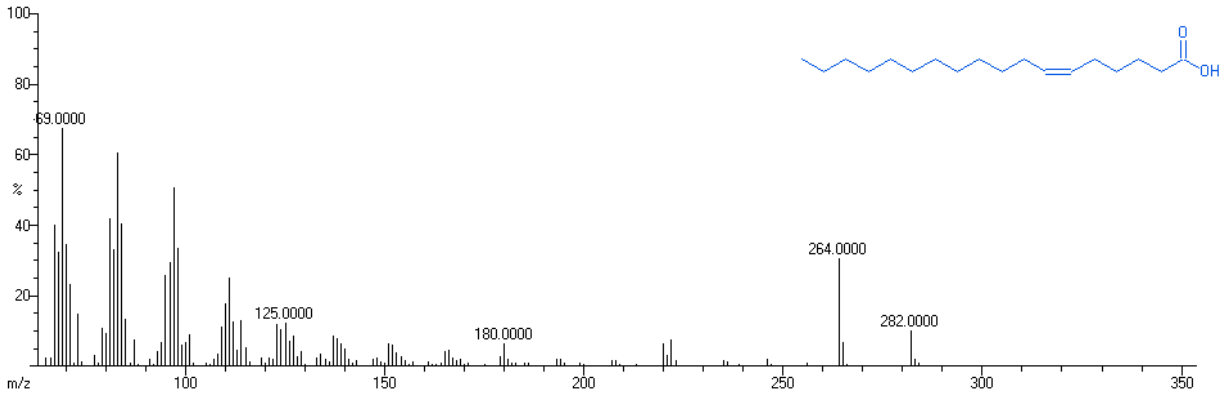

Figure: 2H Mass spectrum of 6-octadecenoic acid, [z]-. (RT: 18.73)

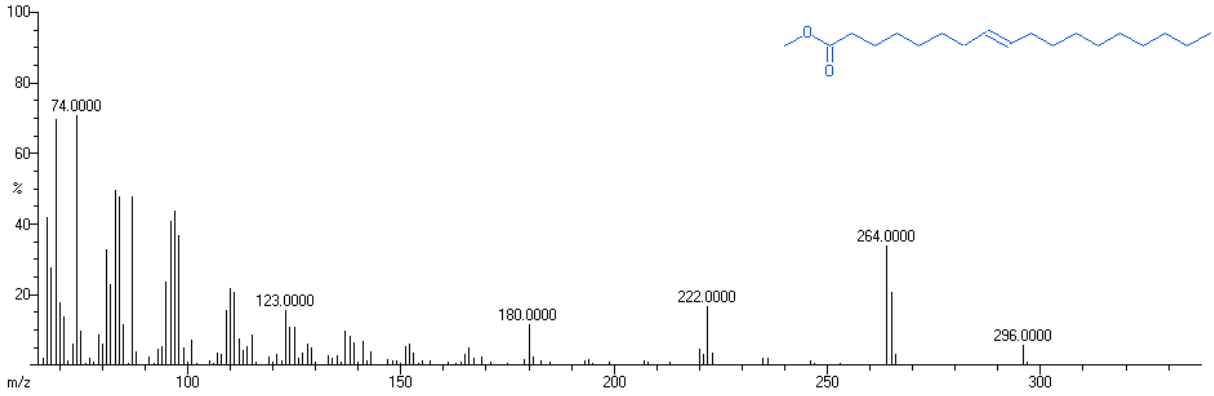

Figure: 2I Mass spectrum of 8-octadecenoic acid, methyl ester. (RT: 17.95)

DISCUSSION

In the present study, the GC-MS analysis of the ethanolic extract of plant of *Indigofera suffruticosa* leaves showed the presence of nine compounds. In terms of percentage amounts 6-octadecenoic acid[z]-, 8-octadecenoic acid, methyl ester, oleic acid, 9-octadecenoic acid[z]-,2-hydroxy-1-[hydroxyl methyl]ethyl ester and n-hexanedecanoic acid were predominant in the extract. These five major compounds have all shown to have cancer preventive, insectifuge, antioxidant, hypochloesterolemic, nematicide, pesticide, lubricant, antiandrogenic, haemolytic, 5-Alpha reductase inhibitor activity. Antioxidant and antimicrobial are shown by pentadecanoic acid-,14-methyl-,methyl ester and octadecanoic acid, 7-hydroxy-, methyl ester. There is growing awareness in correlating the phytochemical compounds and their biological activities [7-9]. *Indigofera suffruticoa* plant is used in Ayurvedic medicine. We report the presence of some of the important components resolved by GC-MS analysis and their biological activities. Thus this type of GC-MS analysis is the first step towards understanding the nature of active principles in this medicinal plant and this type of study will helpful for further detailed study.

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