

## PHYTOCHEMICAL SCREENING AND GAS CHROMATOGRAPH-MASS SPECTROMETER PROFILING IN THE LEAVES OF *SOLANUM INCANUM* L.

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### ABSTRACT

**Objective:** In the present study, the phytochemical constituents of *Solanum incanum* L. leaves have been evaluated by qualitative and gas chromatograph-mass spectrometer (GC-MS) method.

**Methods:** The phytochemicals present in the petroleum ether, methanol, ethanol extract of *S. incanum* L. leaves was investigated by using GC Shimadzu QP2010 system and GC interfaced to a MS equipped with Elite-1 fused silica capillary column. The compounds were separated by using helium as the carrier gas at a constant flow rate of 1.51 ml/minutes. Software adopted to handle mass spectra and chromatograms were GC-MS solution ver. 2.53.

**Results:** Preliminary studies showed the presence of carbohydrates, coumarins, flavonoid, phenol, steroids, and phytosteroids. In the GC-MS analysis of petroleum ether, ethanol, and methanol extract of *S. incanum* L. reported the presence of 12 components. The major chemical constituents are docosahexaenoic acid, 1,2,3 propanetriyl ether (retention time [RT] 9.18), ergoline-8-carboxylic acid, 10-methoxy-6-methyl-methyl ester, (8a) (RT 10.63), ascorbic acid 2,6-dihexadecanoate (RT 11.9), propanoic acid, 2-(3-acetoxy-4,4, 14 trimethylandro-8-en-17-yl) (RT 12.44).

**Conclusion:** This type of GC-MS analysis is the first step towards understanding the nature of active principles in this plant. The isolation of individual bioactive components and their biological activity are necessary for future studies and help to find new drugs.

**Keywords:** Gas chromatograph-mass spectrometer profiling, Phyto components, *Solanum incanum* L., Ascorbic acid 2,6-dihexadecanoate.

### INTRODUCTION

*Solanum incanum* L. belongs to the family *Solanaceae*. Solanaceae family of flowering plants that contains a number of important agricultural crops. They are herbs, shrubs or trees comprising of 85 genera and about 2800 species. They are creeping in nature. The leaves are alternately arranged, usually simple and lack stipules. The flowers are actinomorphic or only slightly zygomorphic in nature. *S. incanum* is used in Eastern and Southern Asia for the treatment of skin diseases and venereal infections. Traditional cures and plant-based remedies remain the main solution to health problems in many developing countries [1].

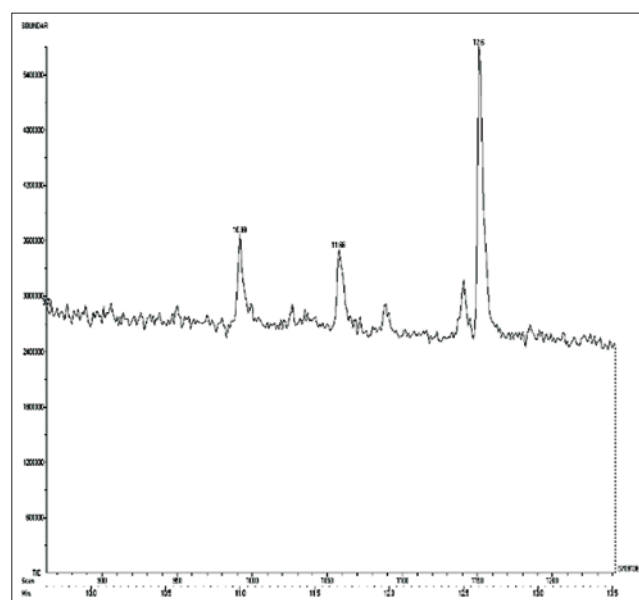
In Southern Africa, the plant has been found to be effective in the treatment of a variety of external benign tumors in veterinary practice [2]. The fruit is a berry or a capsule and it is used for the treatment of toothache, sore throat, and chest complaint [3]. Though the *S. incanum* is extensively used for pain and fever management much of the study has centered on its anti-microbial [4] and anti-tumor [5,6]

effects. It has been used traditionally in treating throat disorders, angina, stomach-ache, colic, headache, painful menstruation, liver pain, and rheumatism. Phytochemical analysis about this plant leaves has not been published. Knowledge of the chemical constituents of the plant is helpful in the discovery of therapeutic agent as well as new sources of economic materials like oil and gums. The most important bioactive constituents of the plants are tannins, alkaloids, flavonoids, and phenolic compounds. Large number of plant species had been

**Table 1: Qualitative phytochemical screening of various extracts of *S. incanum***

S.No	Phytochemical test	PE	C	M	EA
1	Alkaloids	-	-	-	-
2	Carbohydrates	-	-	+	+
3	Glycosides	-	-	-	+
4	Saponins	-	-	-	-
5	Phytosterols and triterpenoids	+	-	-	-
6	Phenol	-	-	-	-
7	Proteins and amino acids	-	-	-	-
8	Tannins	-	-	-	-
9	Flavonoids	+	+	+	-
10	Coumarins	+	+	-	-

*S. incanum*: *Solanum incanum*, PE: Petroleum ether, C: Chloroform, M: Methanol, EA: Ethyl alcohol, -: Negative, + Positive



**Fig. 1: Gas chromatograph-mass spectrometer analysis of petroleum ether extract of *Solanum incanum* L.**

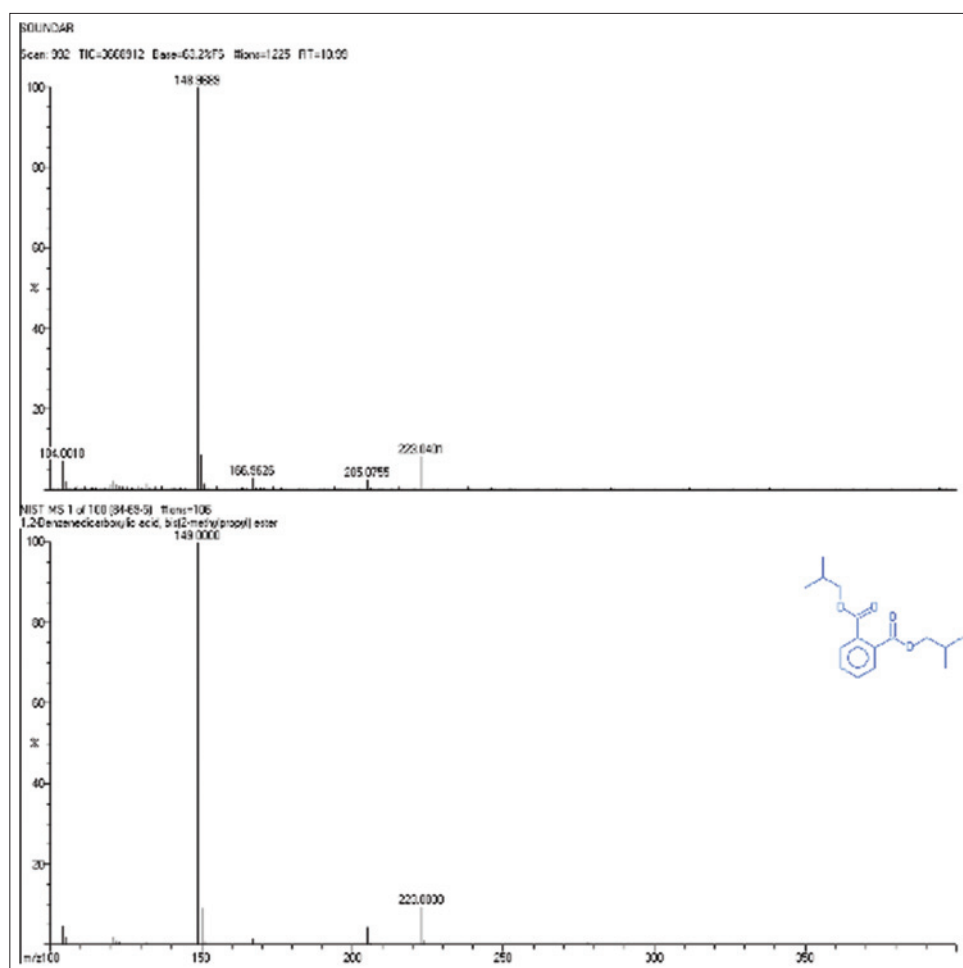


Fig. 2: Gas chromatograph-mass spectrometer analysis of the major peak (1,2Benzenedicarboxylic acid, bis-(2-methylpropyl) ester)

Table 2: Phytocomponents identified in the petroleum ether, ethanol, and methanol leaf extract of *S. incanum* L. by GC-MS

S. no.	RT	Compound name	Molecular formula	MW (g/mol)
<b>Phytocomponents identified in the petroleum ether leaf extract of <i>S. incanum</i> L by GC-MS</b>				
1	10.99	1,2Benzenedicarboxylic acid, bis- (2-methylpropyl) ester	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	278.34
2	11.66	Dibutyl phthalate	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	278.35
3	12.6	Oxirane, tetradecyl	C <sub>16</sub> H <sub>32</sub> O	240.42
<b>Phytocomponents identified in the ethanol leaf extract of <i>S. incanum</i> L. by GC-MS</b>				
4	8.72	3,4 Dimethyl 5oxo 2,5 dihydro 11 pyrrole (4,4 dimethyl 5 (2,3 dimethyl 5 methyl thio 3,4 dihydro 2H pyrrol 2-yl methylene pyridine thioacetic acid 9 ter butyl ester	C <sub>26</sub> H <sub>41</sub> S <sub>2</sub> O <sub>2</sub> N <sub>3</sub>	266.21
5	9.18	Docosaheptaenoic acid, 1,2,3 propanetriyl ether	C <sub>69</sub> H <sub>98</sub> O <sub>6</sub>	1023.51
6	10.14	Propanoic acid	C <sub>3</sub> H <sub>6</sub> O <sub>2</sub>	74.07
7	11.35	Hydroxymethyl colchicines	C <sub>23</sub> H <sub>27</sub> NO <sub>7</sub>	429.46
8	12.44	Propanoic acid, 2-(3-acetoxy-4,4, 14 trimethylandro-8-en-17-yl)	C <sub>27</sub> H <sub>42</sub> O <sub>4</sub>	430.61
<b>Phytocomponents identified in the methanol leaf extract of <i>S. incanum</i> L by GC-MS</b>				
9	10.63	Ergoline-8-carboxylic acid, 10-methoxy-6-methyl-methyl ester, (8a)	C <sub>16</sub> H <sub>16</sub> N <sub>2</sub> O <sub>2</sub>	268.31
10	11.9	Ascorbic acid 2,6-dihexadecanoate	C <sub>38</sub> H <sub>68</sub> O <sub>8</sub>	652.94
11	13.18	2 (3H)-Furanone, dihydro-5-tetradecyl	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282.52
12	13.57	Dasycarpidan-1-methanol, acetate (ester)	C <sub>20</sub> H <sub>26</sub> N <sub>2</sub> O <sub>2</sub>	326.43

*S. incanum*: *Solanum incanum*, RT: Retention time, GC-MS: Gas chromatograph-mass spectrometer

screened for their pharmacological properties, but still a vast wealth of endangered species are unexplored. The medicinal plants are at interest to the field of biotechnology, as most of the drug industries depend on plants for the production of pharmaceutical compounds. Hence, the present investigation is the attempt in this direction including the determination of qualitative phytochemical and gas chromatograph-mass spectrometer (GC-MS) analysis of *S. incanum* L. leaves.

## METHODS

### Authentication of plant

Different parts of *S. incanum* L. including roots, stems, leaves, and flowers, were collected from a locality in Tenkasi (Tamil Nadu). The sample was authenticated by the Botanical Survey of India (BSI), Ministry of Environmental and Forests, Tamilnadu Agriculture University Campus, Coimbatore and voucher No-BSI/SRC/5/23/2010-11/Tech/1605.

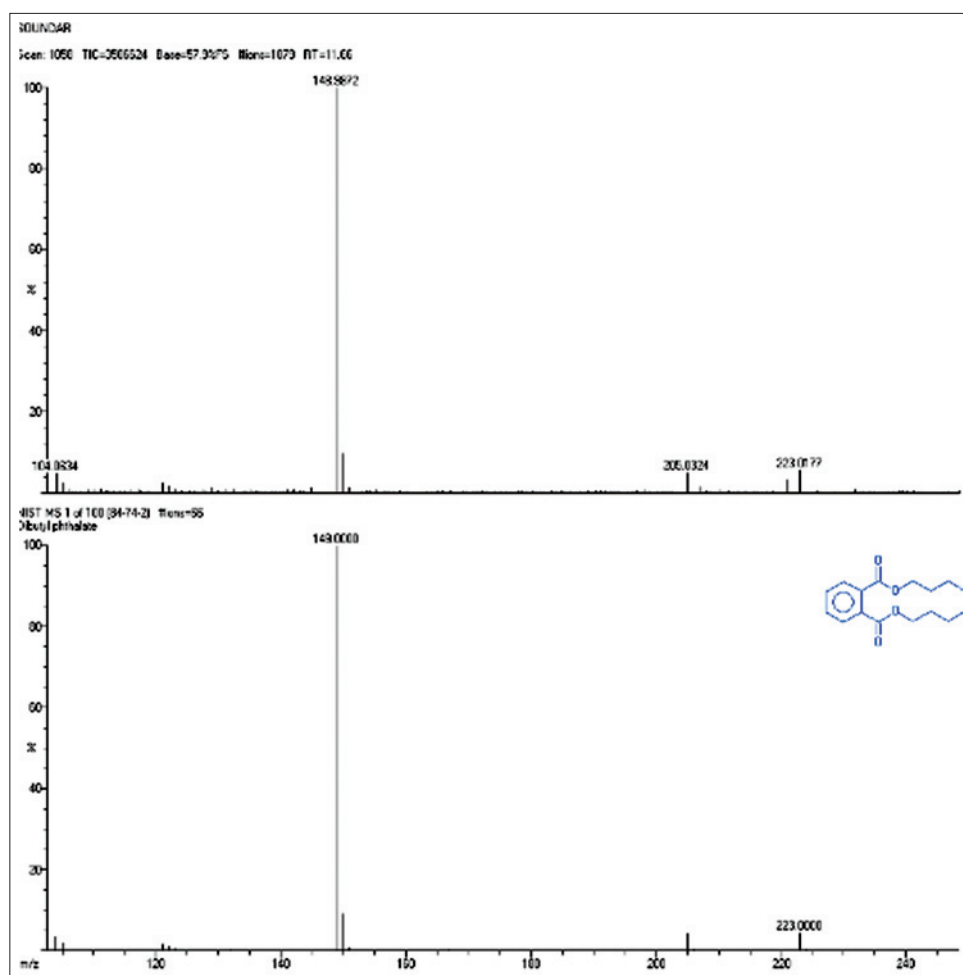


Fig. 3: Gas chromatograph-mass spectrometer analysis of the major peak (dibutyl phthalate)

#### Extraction and drying

About 10 g of dry sample powder was weighed and macerated with 40 ml of each solvent (petroleum ether, chloroform, methanol, and ethanol) separately and kept overnight in a shaker. The extract was collected after filtration using Whatman No.1 filter paper and was stored. Another 40 ml of solvent was added to the residual mixture and incubated in a shaker for 24 hrs and the extract was collected. This procedure was repeated once again and the extract was evaporated below 40°C, which was used for further phytochemical analysis [7].

#### Yield

The yield obtained from 10 g of extract:

Petroleum ether: 0.291 g

Methanol: 0.576 g

Chloroform: 0.348 g

Ethanol: 0.723 g

#### Procedure for phytochemical screening

Phytochemical examinations were carried out for all the extracts, as per the standard methods [8,9].

#### Preparation of extracts for GC-MS analysis

The sample was dried and pulverized to a powder in a mechanical grinder. Required quantity of the leaf powder of *S. incanum* L. was weighed transferred to a flask, treated with the petroleum ether until the powder was fully immersed, incubated overnight, and strained through a Whatman No.41 filter paper. The filtrate is then concentrated to 1 ml by bubbling nitrogen gas into the solution and 2 µl sample of the solution was employed in GC-MS for analysis of different compounds.

#### GC-MS analysis of phytochemicals

A very powerful combination results from the union of the high-resolution capacity of GC with the identification capabilities of mass spectrometry. The resulting data have a tri-dimensional nature, from which retention times (RT), chromatographic areas, and mass spectra can be obtained for every individual element of a complex variety. Petroleum ether, ethanol, and methanol extract of *S. incanum* L. was analyzed by GC-MS method. GC-MS technique was done by using GC Shimadzu QP2010 system and GC interfaced to a MS. It is equipped with Elite-1 fused silica capillary column. Helium gas (99.99%) was employed as the carrier gas at a constant current rate of 1.51 cc/minute and an injection volume of 2 µl was employed (split ratio: 20). Injection temperature was 200°C; ion source temperature 200°C. The oven temperature was programmed from 70°C (isothermal for 2 minutes), with an increase of 3000°C for 10 minutes. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds with a scan range of 40-1000 m/z. Total GC running time was 35 minutes.

#### Identification of components

Interpretation of mass spectra of GC-MS was performed utilizing the database of National Institute Standard and Technology (NIST) having more than 62,000 designs. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight (MW), and structure of the components of the test materials were ascertained.

#### RESULTS AND DISCUSSION

To explore the importance of any medicinal plant, the initial step is to screen for its phytochemicals because it provides a broad plan concerning the nature of compounds present in it. In the present study, the leaves of

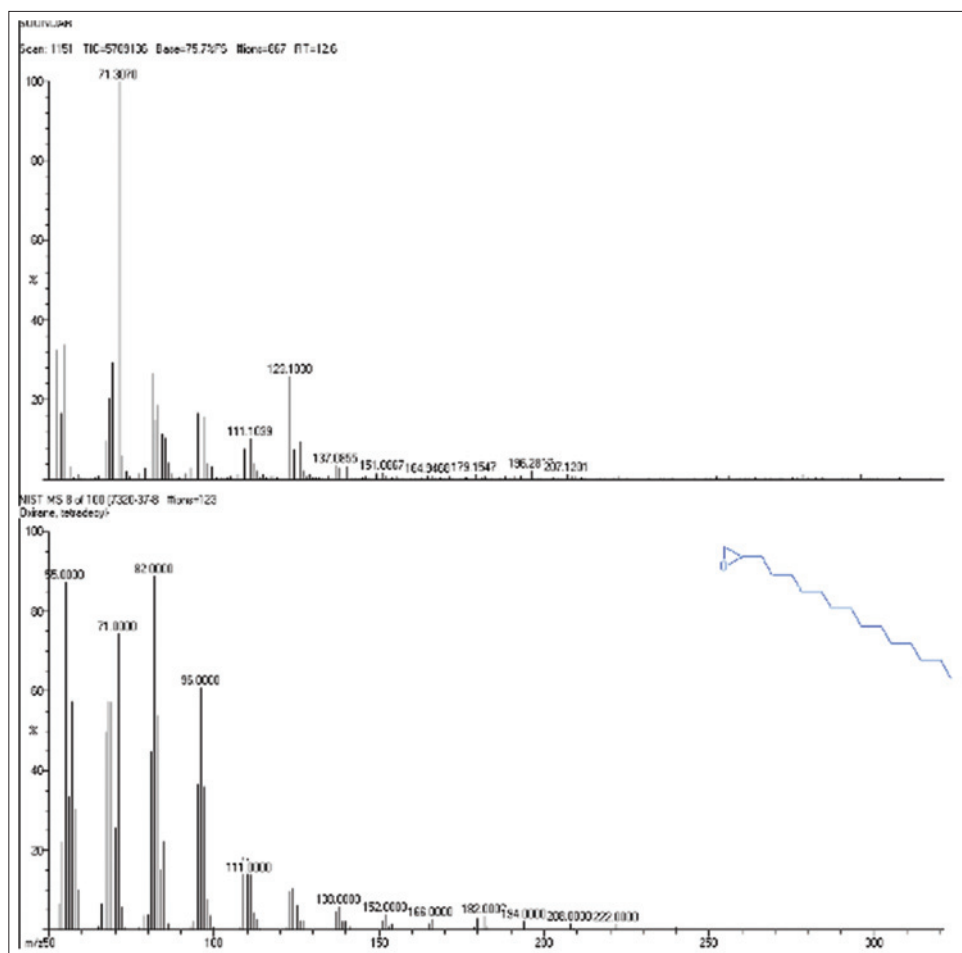


Fig. 4: Gas chromatograph-mass spectrometer analysis of the major peak (oxirane, tetradecyl)

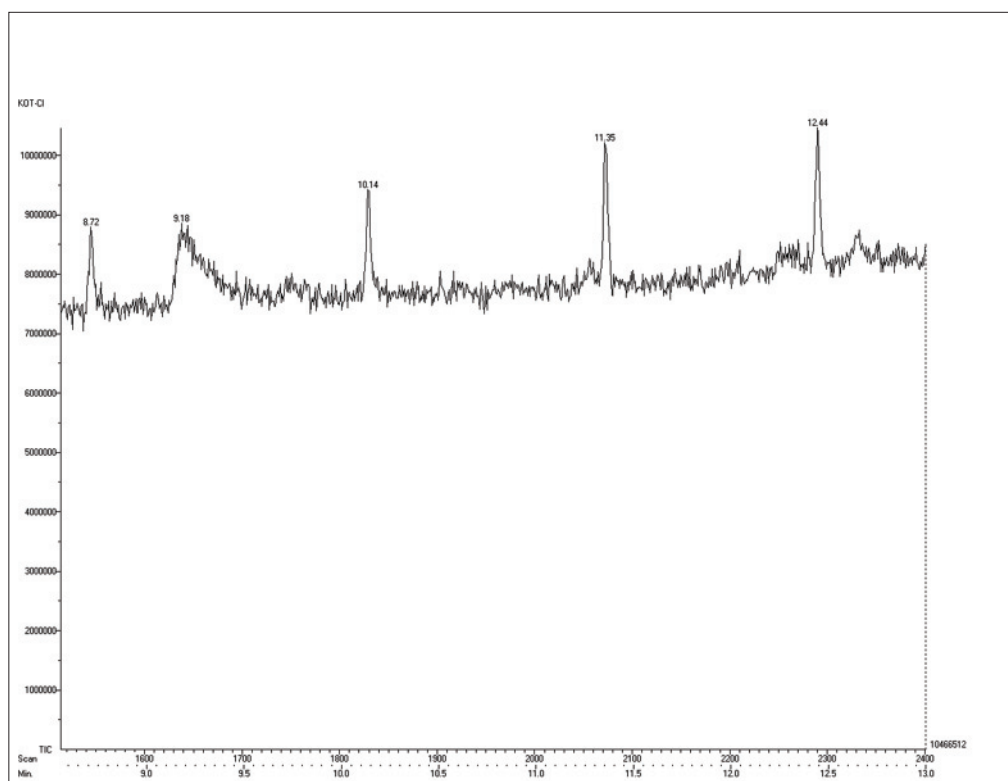


Fig. 5: Gas chromatograph-mass spectrometer analysis of ethanol extract of *Solanum incanum* L.

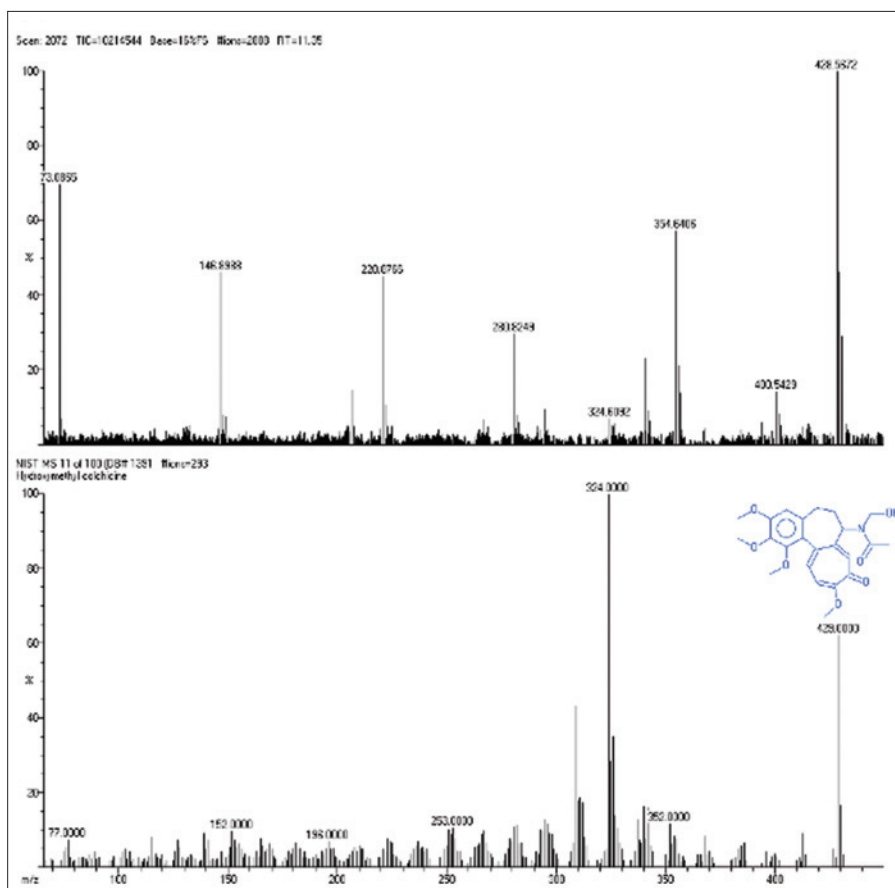


Fig. 6: Gas chromatograph-mass spectrometer analysis of the major peak (hydroxymethyl colchicines)

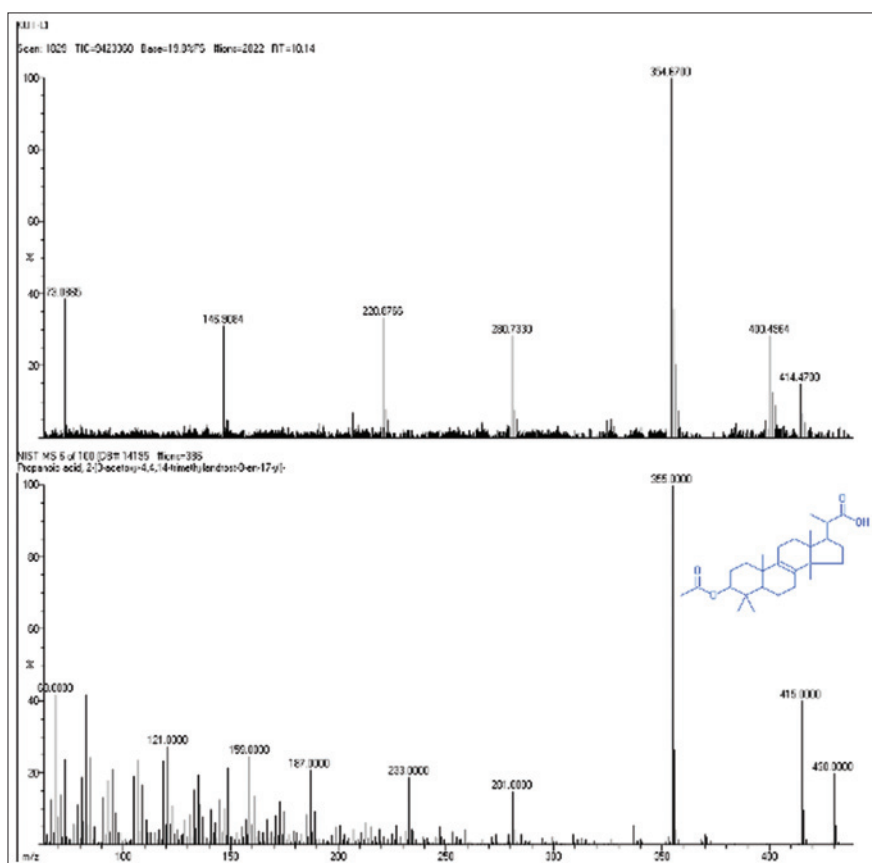


Fig. 7: Gas chromatograph-mass spectrometer analysis of the major peak (propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrosta-8-en-17-yl))

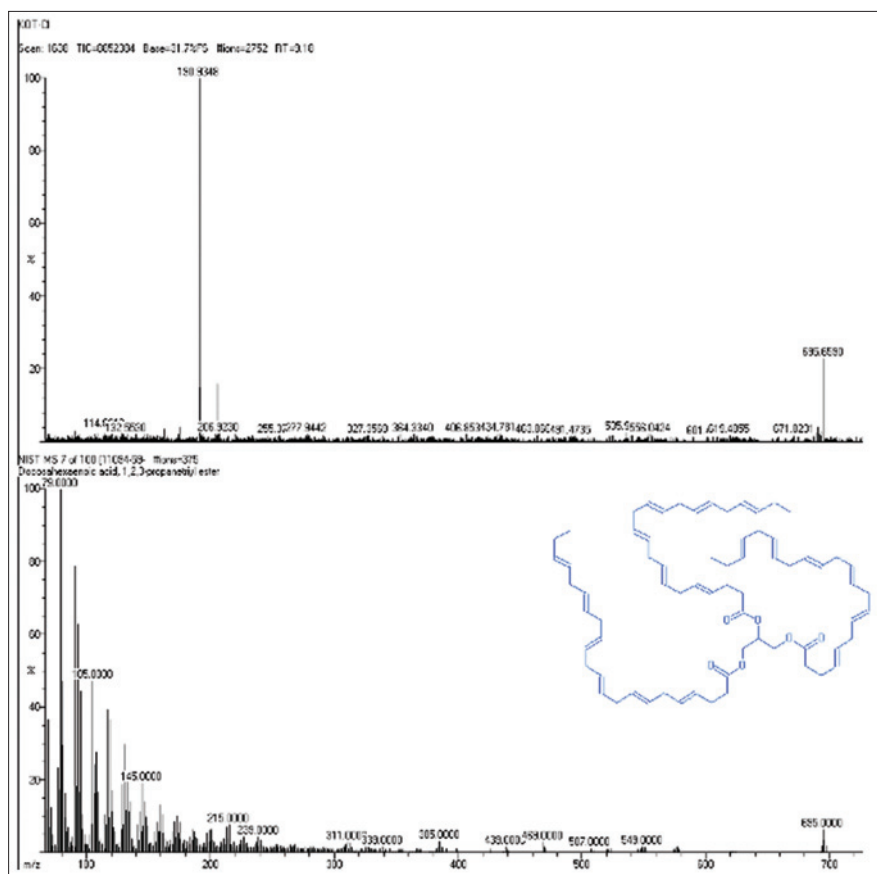


Fig. 8: Gas chromatograph-mass spectrometer analysis of the major peak (docosahexaenoic acid, 1,2,3 propanetriyl ether)

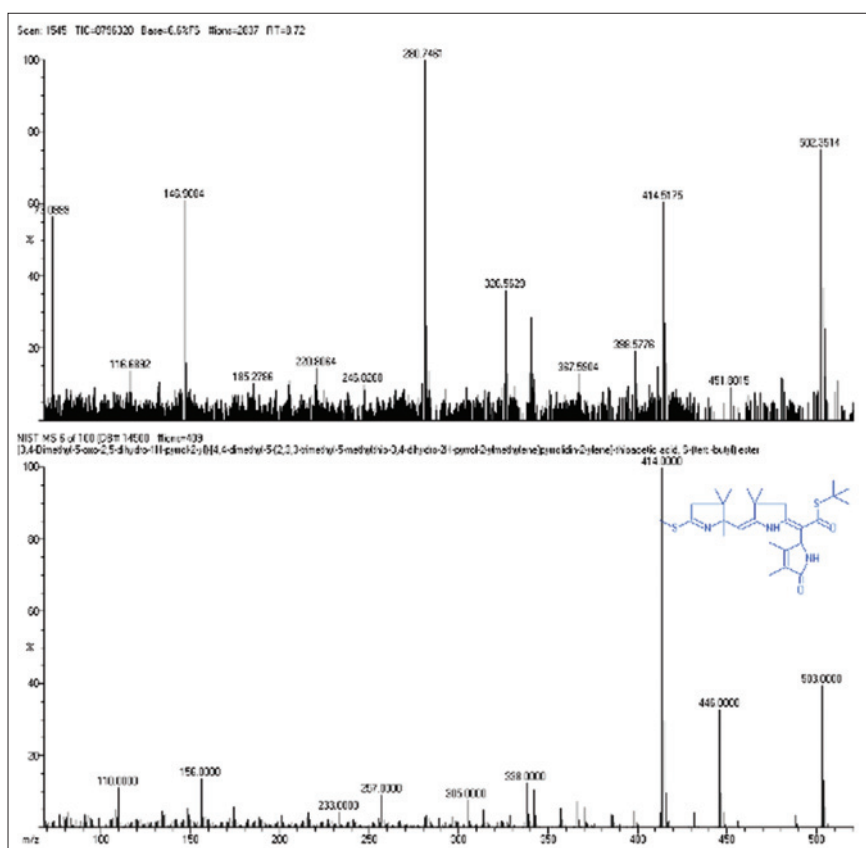


Fig. 9: Gas chromatograph-mass spectrometer analysis of the major peak (3,4 Dimethyl 5oxo 2,5 dihydro 11 pyrrole(4,4 dimethyl 5(2,3 dimethyl 5 methylthio 3,4 dihydro 2H pyrrol 2-yl) methylene pyroldine thioacetic acid 9 ter butyl ester)

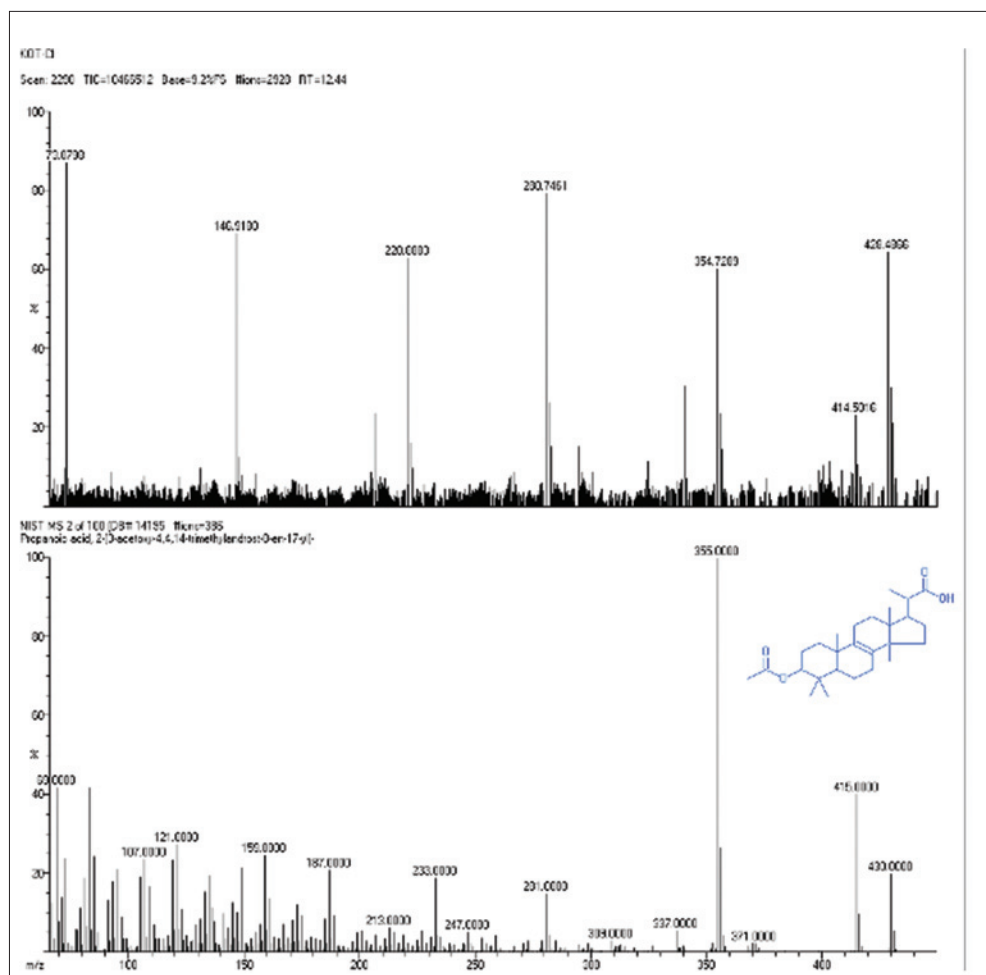


Fig. 10: Gas chromatograph-mass spectrometer analysis of the major peak (propanoic acid, 2-(3-acetoxy-4,4, 14 trimethylandrosta-8-en-17-yl))

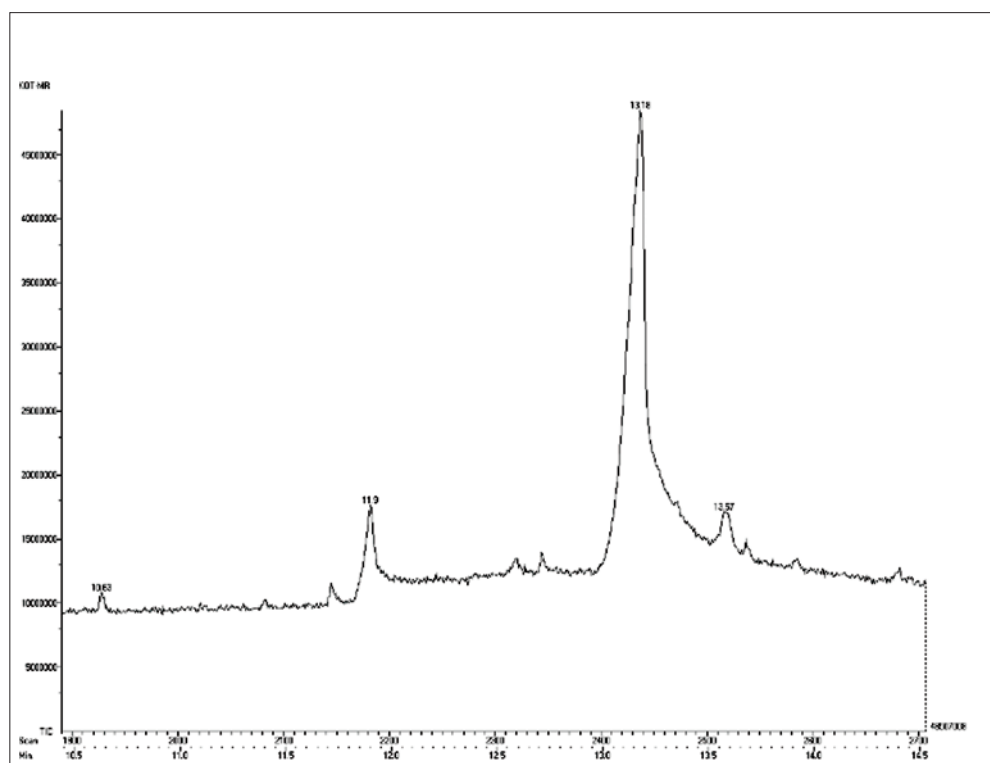


Fig. 11: Gas chromatograph-mass spectrometer analysis of methanol extract of *Solanum incanum* L.

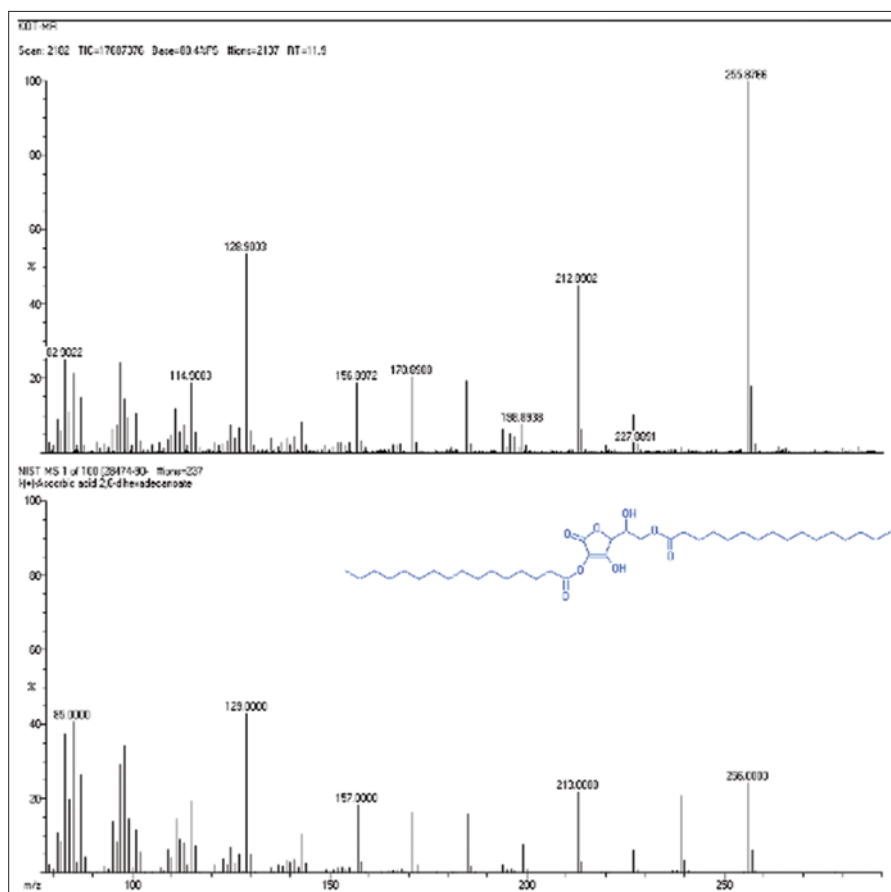


Fig. 12: Gas chromatograph-mass spectrometer analysis of the major peak (ascorbic acid 2,6 dihexadecanoate)

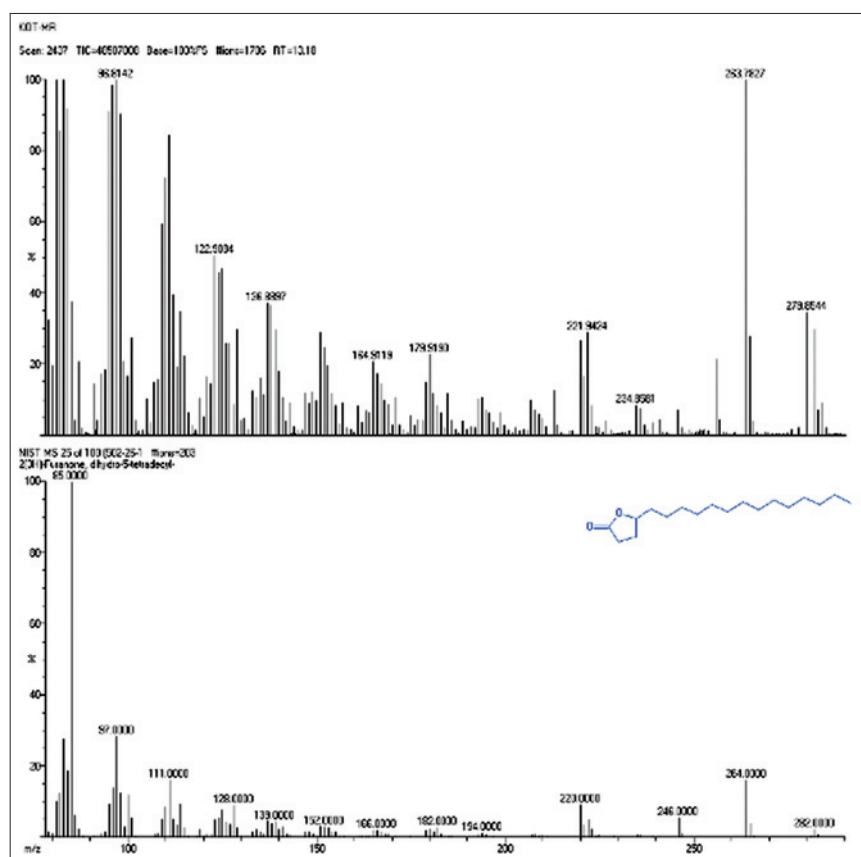


Fig. 13: Gas chromatograph-mass spectrometer analysis of the major peak (2(3H)-Furanone methanol, acetate (ester))



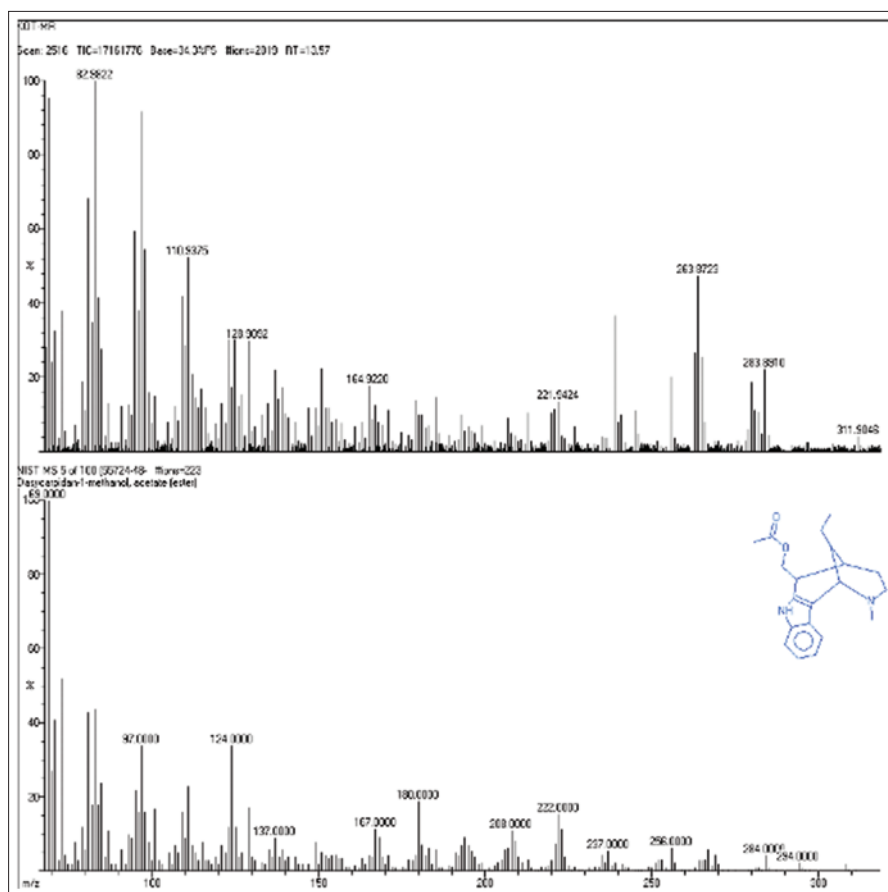


Fig. 14: Gas chromatograph-mass spectrometer analysis of the major peak (Dascarpidan-1- dihydro-5-tetradecyl)

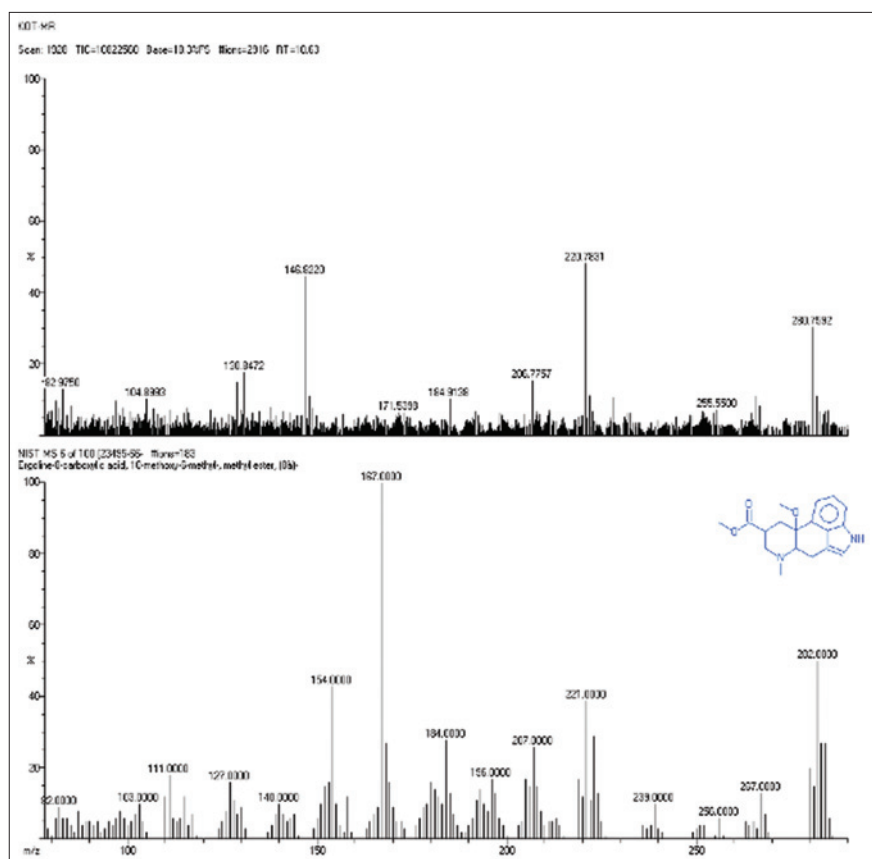


Fig. 15: Gas chromatograph-mass spectrometer analysis of the major peak (ergoline-8-carboxylic acid, 10-methoxy-6-methyl-methyl ester, (8a))

*S. incanum* L. were preliminarily screened for the phytochemicals, among the four extracts, petroleum ether extract was found to be rich in all the phytoconstituents followed by methanol extract. The chloroform and ethanol extract were uniformly positive for the phytochemicals studied. The flavonoid is found to be the major component in this *S. incanum* L. Preliminary phytochemical screening of *S. incanum* L. is shown in the Table 1. The phytoconstituent present in ethanol, methanol, and petroleum ether extract of *S. incanum* L. was identified by GC-MS analysis (Figs. 1-15).

The active compounds with their RT, molecular formula and MW in all three extracts of *S. incanum* L. is presented in Table 2.

Previous studies on the phytochemical screening of aloe vera L also revealed the presence of tannin, saponin, flavonoids, and terpenoids [10]. This phytochemical screening aids as an initial step for future determination of its activity like antioxidant, anticancer, anti-inflammatory. In a previous report on phytochemical screening of *strychnos Nux-vomica* revealed the presence of carbohydrate, alkaloid, tannin, steroid, triterpenoid, and glycoside in the extract [11].

#### CONCLUSION

Totally, 12 compounds were identified in ethanol, methanol, and petroleum ether extracts of *S. incanum* L. Thus, the plant studied can be used as a potential source of a possible supply of latest helpful medication. The phytochemical characterization of the extracts, the isolation of accountable bioactive compounds, and their biological activity are necessary for future studies.

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