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Research article

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Identification of biomarkers present in *Trachyspermum ammi* leaves oil by GC-MS analysis

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ABSTRACT

Trachyspermum ammi is a traditional plant which is native of Egypt and is cultivated in Iraq, Iran, Afghanistan, Pakistan, and India. A GC-MS analysis was performed to identify the biomarkers present in *Trachyspermum ammi* leaves oil. The GC-MS analysis leads to identification of different phytoconstituents present in volatile oil that was extracted from leaves with help of claviger apparatus. The phytoconstituents were found are thymol, o-cymene, caryophyllene, ethyl methyl acetal, alpha, pinene, squalene, delta-cadinene, carvacrol. The result of GC-MS analysis confirmed the presence of these all compounds. The presence of phytoconstituents reveals the medicinal value of leaves.

Keywords: GC-MS analysis, *Trachyspermum ammi* leaves, Volatile oil.

INTRODUCTION

The present global scenario is changing their face towards use of herbal medicine due to lesser side effects. Folk medicinal system has deep rooted history among rural population in India. This unique system of knowledge has evolved from harmonious living of aboriginal people with nature. The majority of people on earth still depend on traditional medicine for every day health care [1-3]. In ancient times before the allopathy came into practice herbal medicine was

only source of treatment. As the field of medicine advances allopathic medicine become popular due to its fast therapeutic actions but have several side effects. Then again phytomedicine gained momentum as it not only cures the ailment but also have no side effects [4].

Trachyspermum ammi is native of Egypt, cultivated in India, Pakistan, Iran and Afghanistan. *Trachyspermum ammi* L. belongs to family Apiaceae. Ajwain is tall, erect, profusely branched annual herb whose stem is striated grooved hairless

or minutely pubescent. The leaves are pinnate, distant and have several lateral divided leaflets. It is suitable to grow in areas where the salt content in the soils is high, mostly in arid and semi-arid regions [5,6]. *Trachyspermum ammi* seeds are used as a spice and as several medicinal properties in treatment of bronchial problems, gastrointestinal ailment [7].

MATERIALS AND METHODS

Collection of plant material

Plant material was collected from Herbal garden, Himayat sagar road, Hyderabad, in the month of November 2016. Leaves were collected and dried before initializing the process. Drying is done under shade for 6-7 days till the leaves were dried completely. The dried leaves of *Trachyspermum ammi* were used for extraction for

volatile oil with help of clavenger apparatus. The volatile oil was used for GC-MS analysis.

Analysis of sample by GC-MS

The sample is injected into GC inlet where it is vaporized and passed onto chromatographic column by the inert Helium carrier gas and the mixture is separated by relative interactions based on molecular weight. The column is placed in the oven which works on temperature gradient that is 30mm long and 0.25 in diameters for optimal separation. Mass spectrometer works as detector where the molecule is bombarded with electrons which break the molecule into ions or molecular fragments. Mass spectrometer is connected to computer data base which gives mass chromatogram and compare it with different chromatograms from the library from around the globe. The peaks in the chromatogram not only identify the molecule but also show its abundance.

Specifications for sample analysis by GC-MS [8, 9]

NAME OF INSTRUMENT	Agilent 6890GC with 5973N MSD
COLUMN	HP5MScolumn×30mmL×0.25mmInternal diameter×0.25 thickness
OVEN TEMPERATURE PROGRAM	
INITIALTEMPERATURE	40°C
HOLDUPTIME	2minutes
RATE	10°C/minute
FINAL TEMPERATURE	280°C
HOLD UP TIME	10 minutes
TOTAL RUN TIME	32 minutes
INJECTION TEMPERATURE	280°C
AUXILLARY TEMPERATURE	280°C
IONISATION SOURCE	230°C
QUADRAPOLE TEMPERATURE	150°C
SOFTWARE	Agilent chemstation
LIBRARY DATABASE	NIST/WILT2Y (IN 1999)
CARRIER GAS	Helium
FLOW RATE	1ml/min
INJECTION MODE	Splitters
SPLIT RATIO	10:1
IONISATION MODE	Electron capture
MASS SCAN RANGE	29-600 Dalton

RESULTS AND DISCUSSION

Qualitative analysis report of ethanolic extract by GC-MS

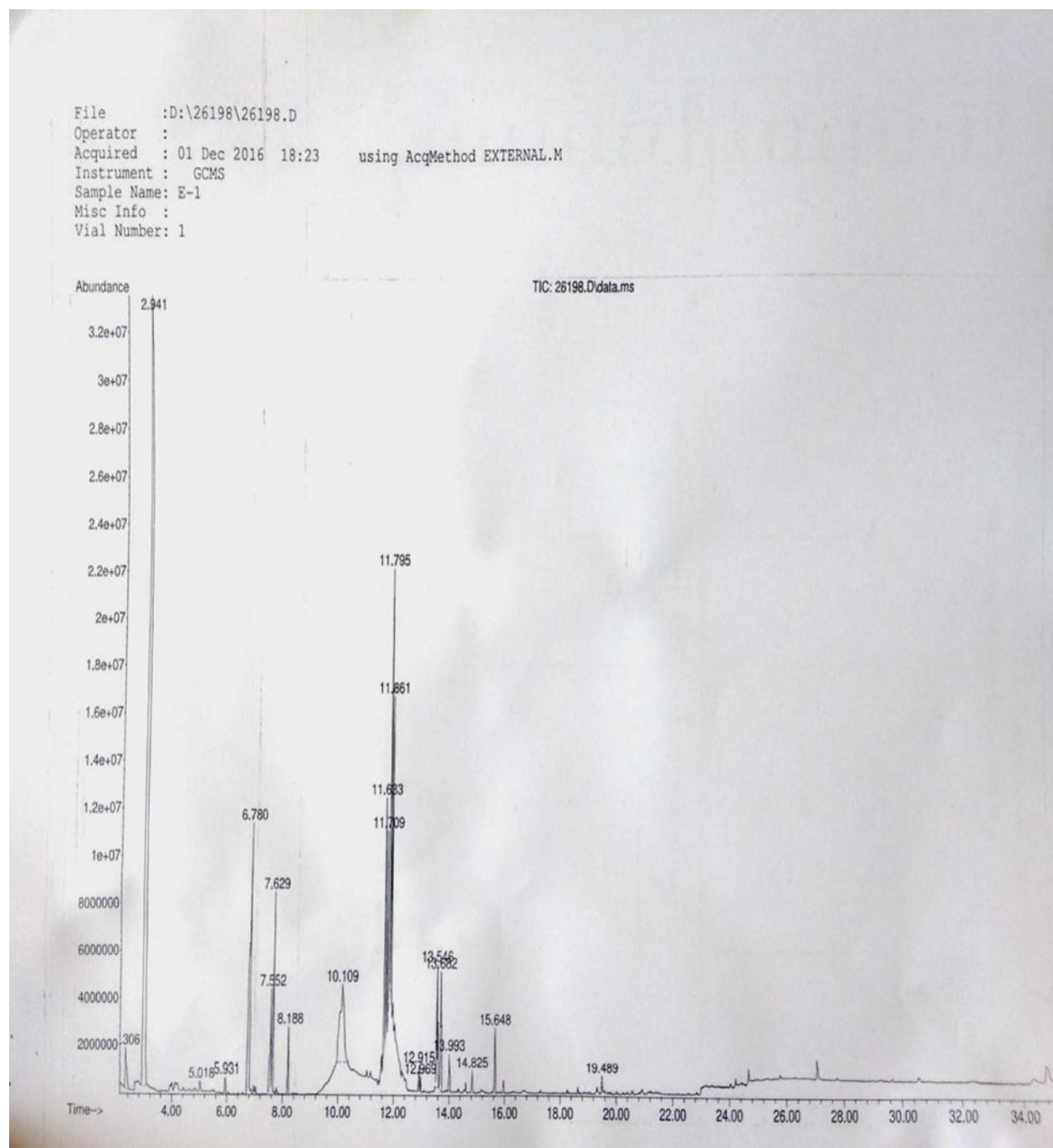


Figure 1: Mass spectrum of leaves oil

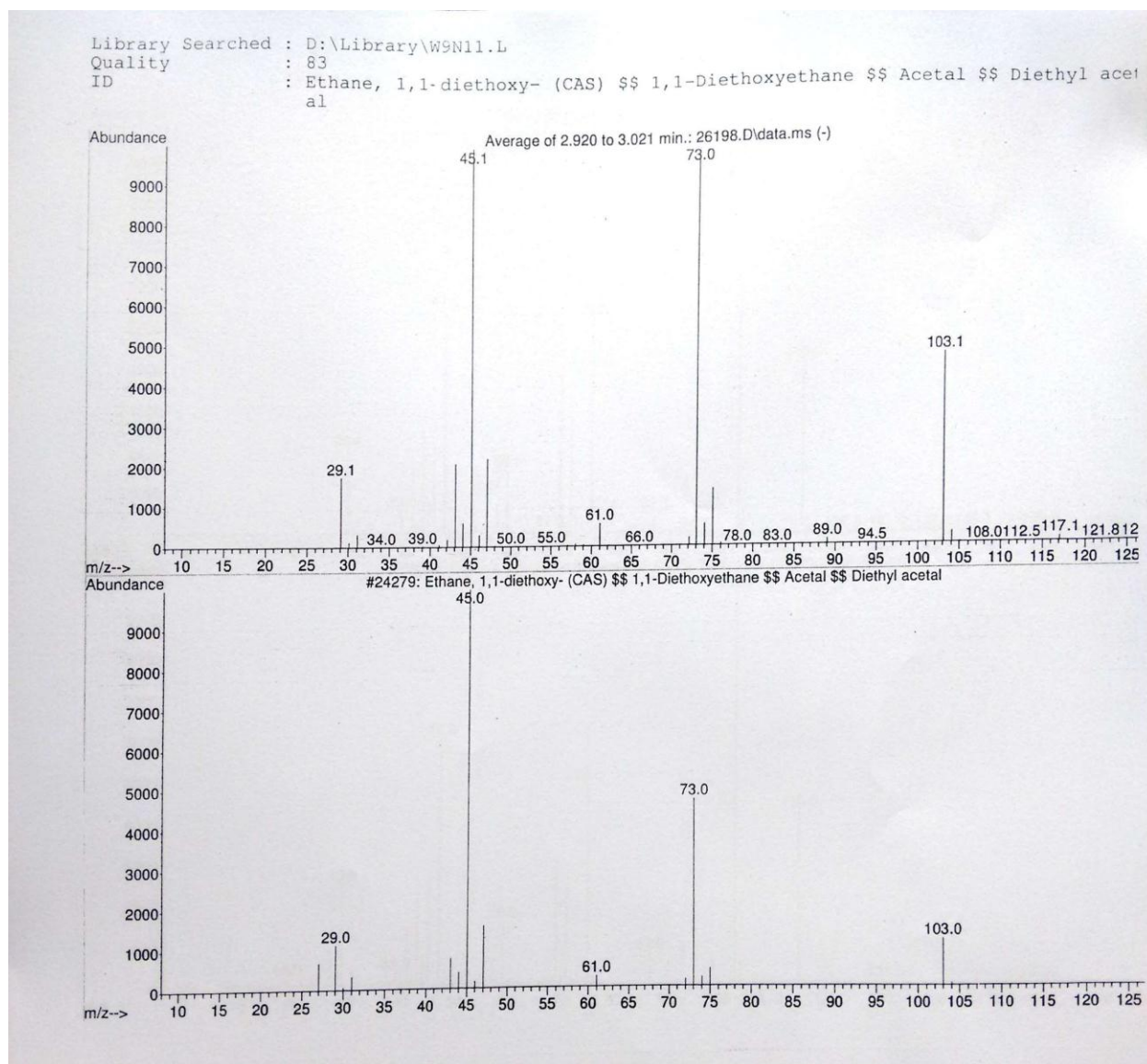
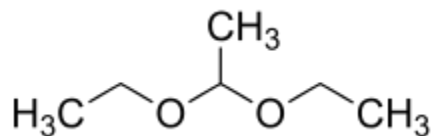


Figure 2: Structure
 Ethane, 1, 1-diethoxy- ; Diethyl acetal



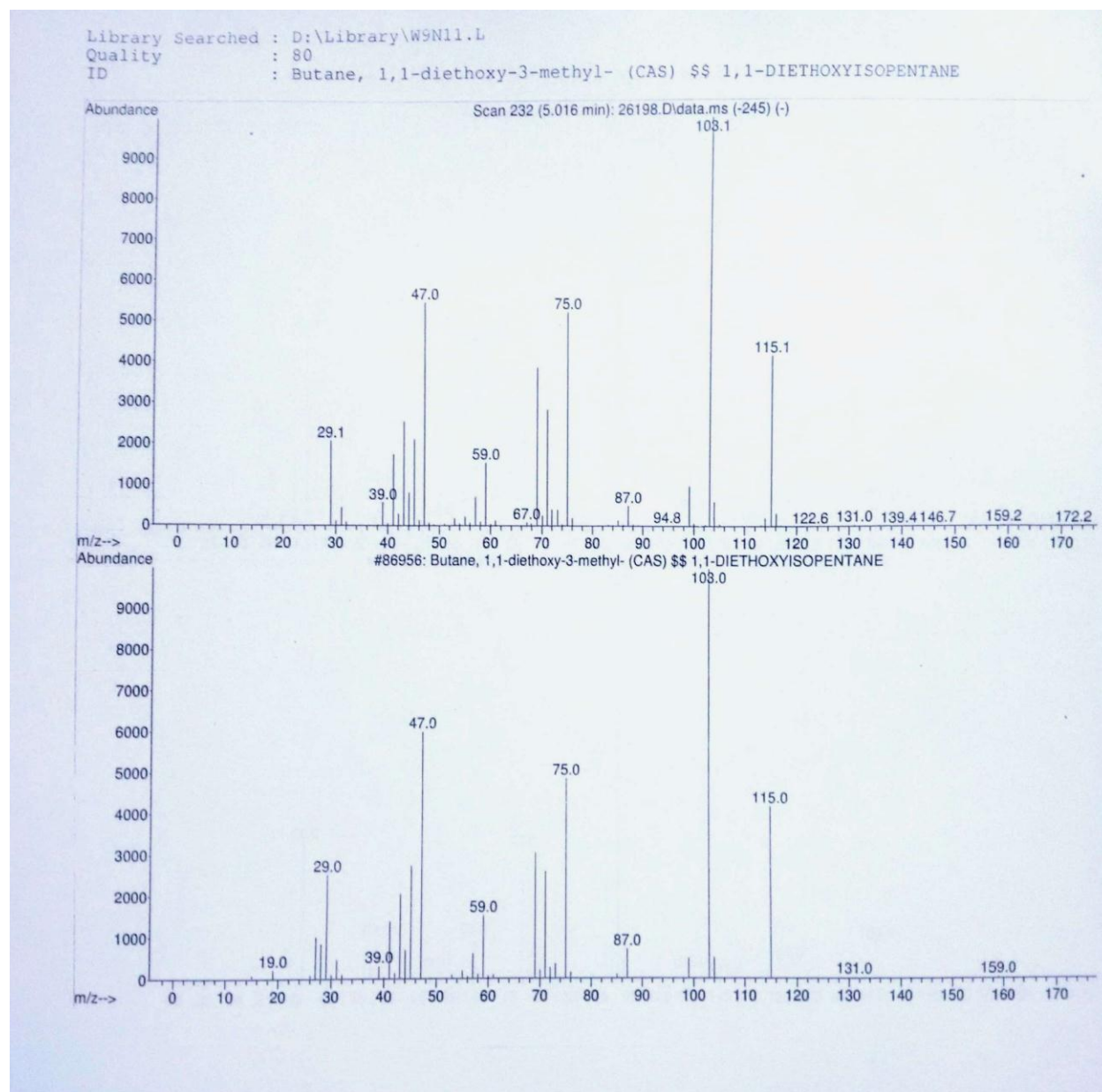
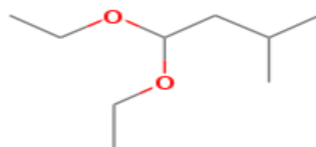


Figure 3: Structure
Butane, 1, 1-diethoxy-3-methyl- (CAS) ; 1,1-Diethoxyisopentane



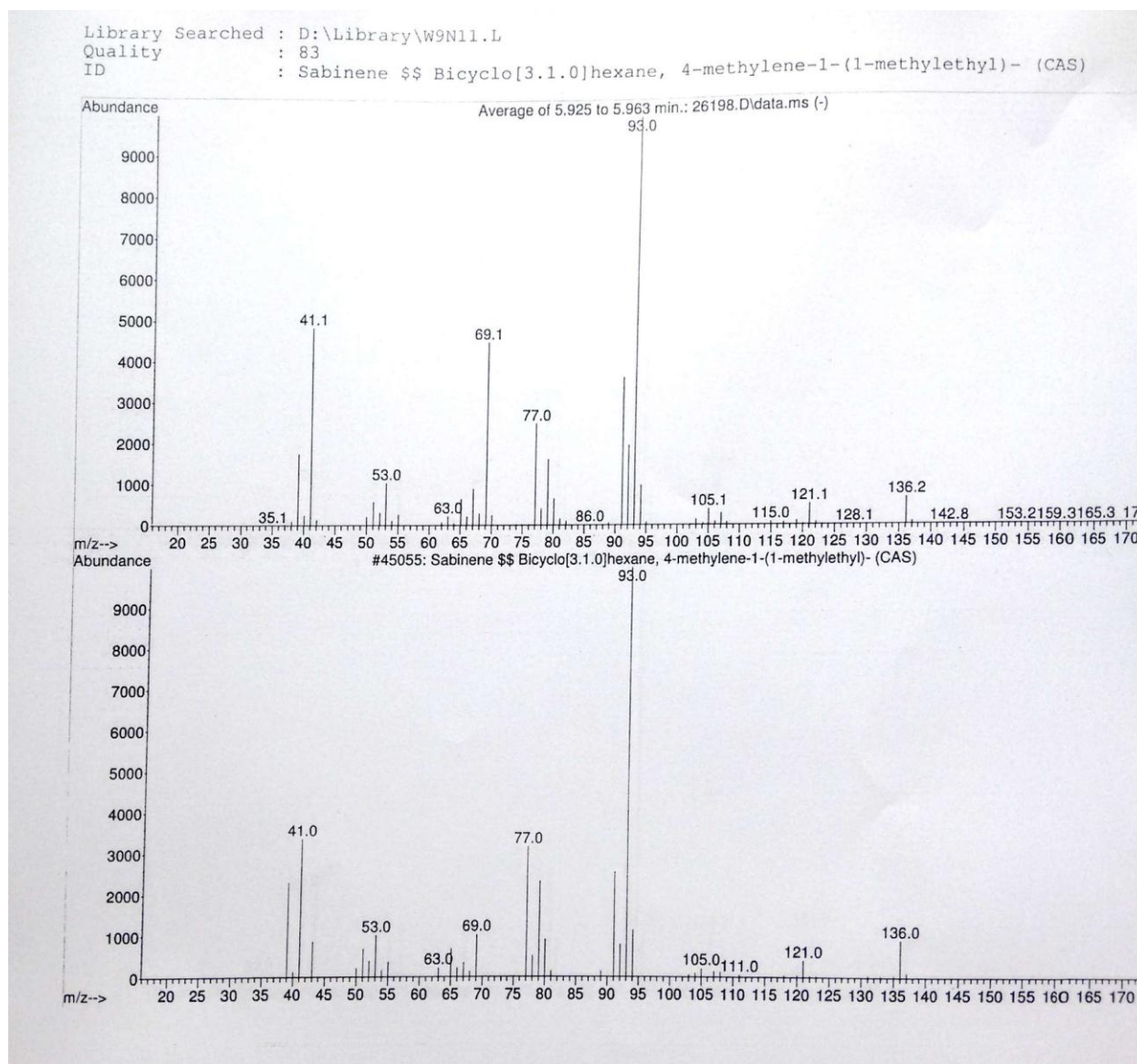
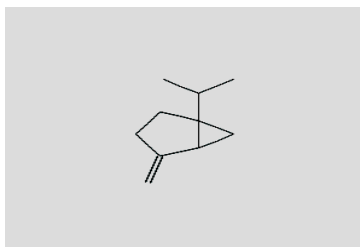
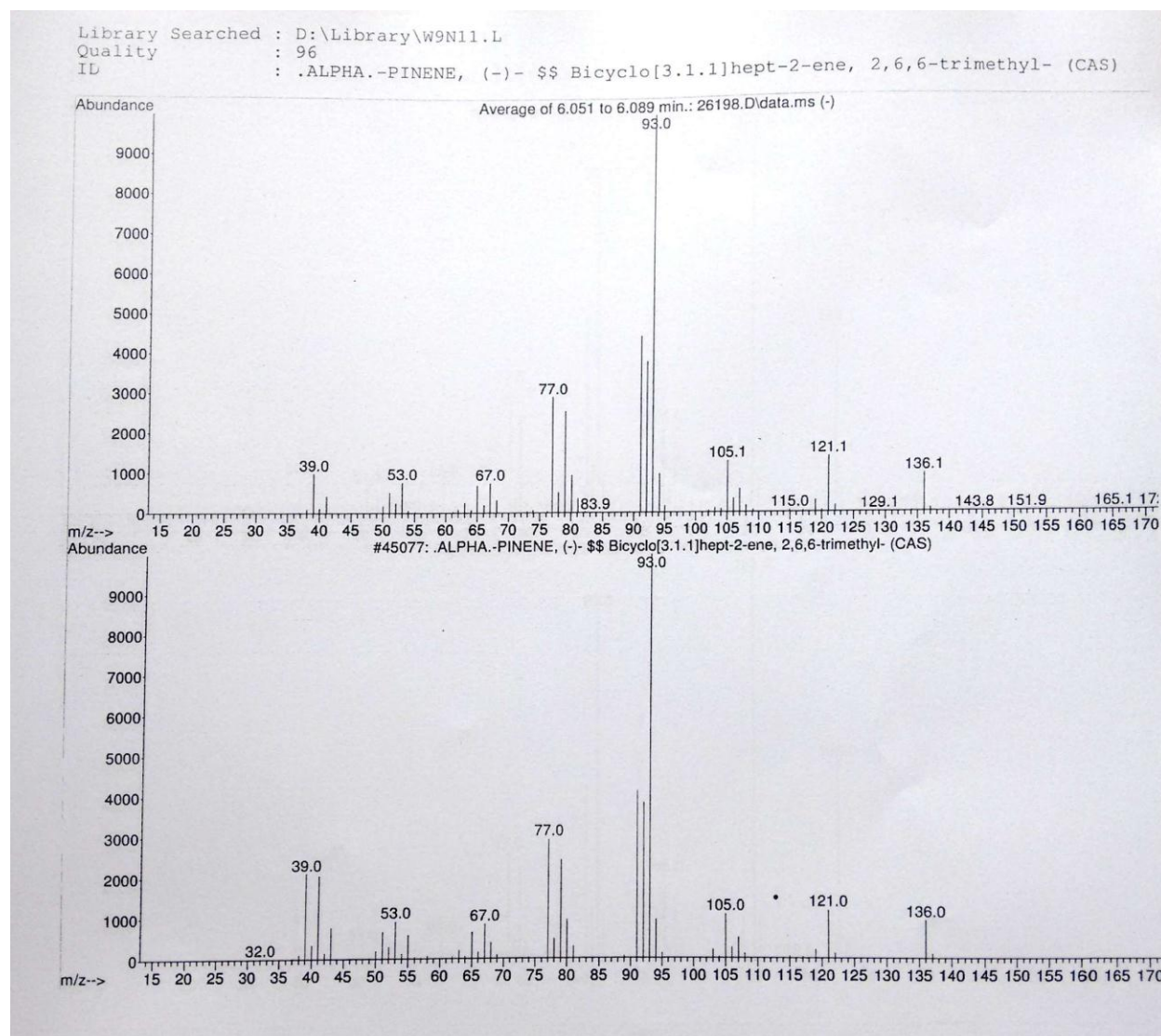
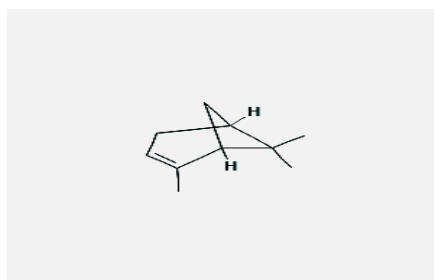


Figure 4: Structure
Sabinene





**Figure 5: Structure
Alpha-Pinene**



Alpha-Terpinene; Para-Mentha-1, 3-Diene

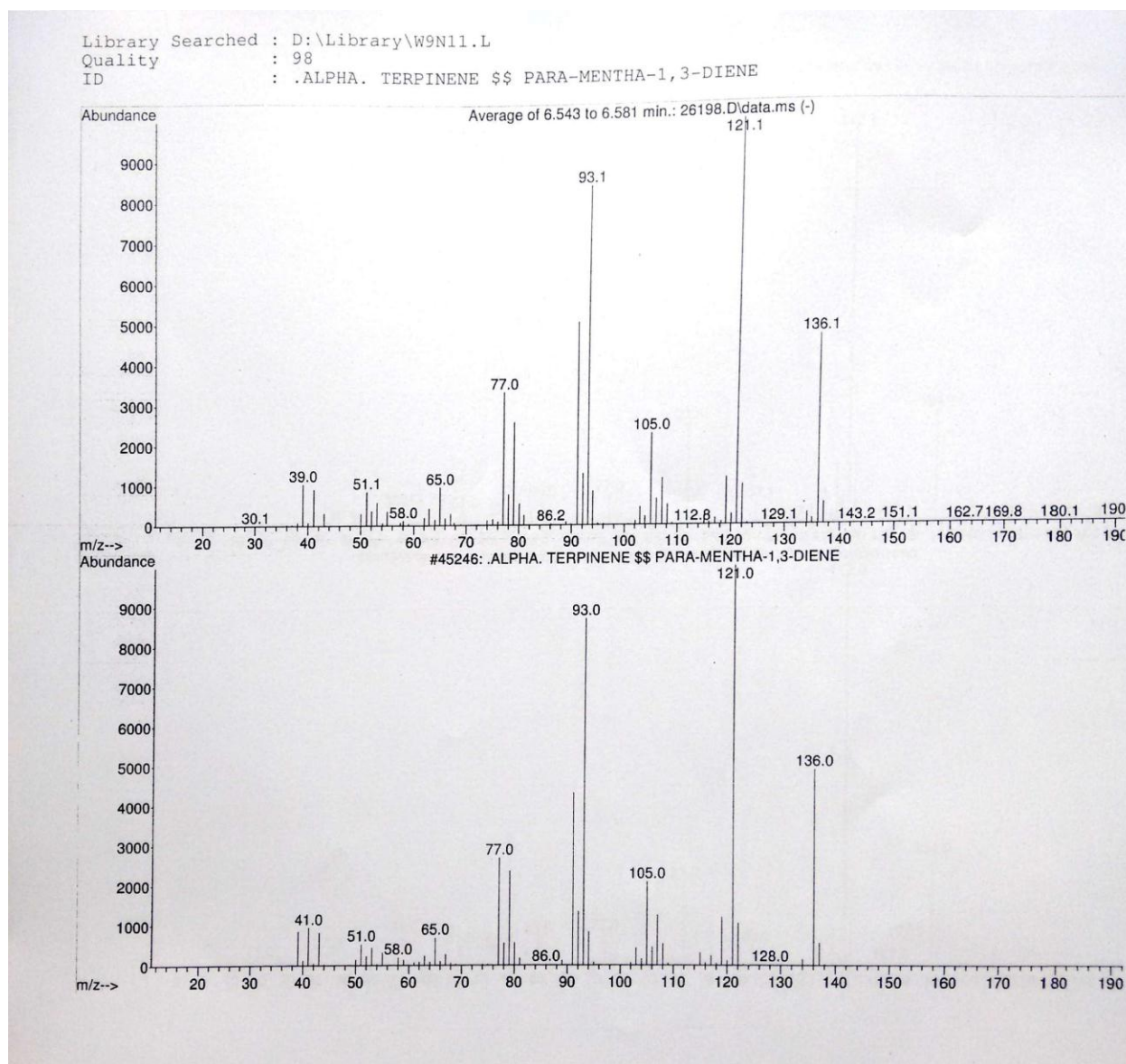
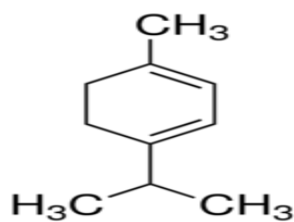


Figure 6: Structure
 Alpha-Terpinene; Para-Mentha-1, 3-Diene



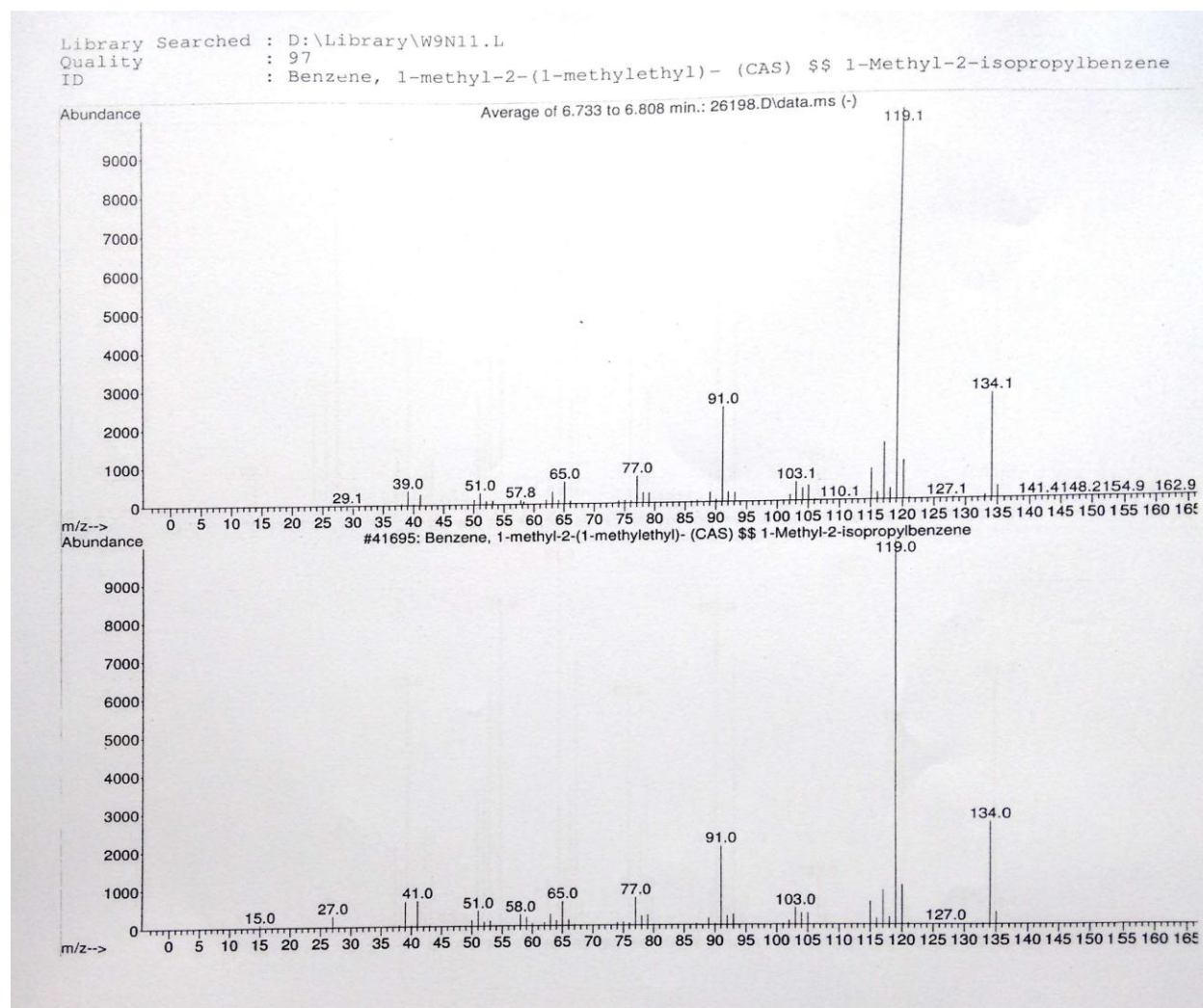


Figure 7: Structure
Benzene, 1-methyl-2-(1-methylethyl)- (CAS) ; 1-Methyl-2-isopropylbenzene

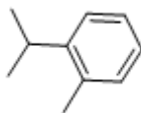


Table 1: Phytoconstituents found in volatile oil of *Trachyspermum ammi* leaves

S. No.	NAME	MOLECULAR FORMULA	MOLECULAR WEIGHT
1	Ethyl methyl acetal	C ₅ H ₁₂ O ₂	104.149
2	Diethyl acetal	C ₆ H ₁₄ O ₂	118.1742
3	1,1-Diethoxyisopentane	C ₉ H ₂₀ O	160.254
4	Sabinene	C ₁₀ H ₁₆	136.238
5	Alpha-Pinene	C ₁₀ H ₁₆	136.238
6	Alpha-Terpinene	C ₁₀ H ₁₈ O	154.2493

7	1-Methyl-2-isopropylbenzene	C ₁₀ H ₁₄	134.2182
8	1,8-Cineole	C ₁₀ H ₁₈ O	154.253
9	Beta-Myrcene	C ₁₀ H ₁₆	136.238
10	Gamma-Terpinene	C ₁₀ H ₁₆	136.238
11	O-Cymene	C ₁₀ H ₁₄	134.2182
12	Glycerol	C ₃ H ₈ O ₃	92.094
13	Thymol	C ₁₀ H ₁₄ O	150.221
14	Thymol;Phenol,5- methyl-2-(1-methylethyl)-	C ₁₀ H ₁₄ O	150.21756
15	Carvacrol	C ₁₀ H ₁₄ O	150.221
16	3-Methyl-4-isopropyl phenol	C ₁₀ H ₁₄ O	150.2176
17	Alfa-Copaene	C ₁₅ H ₂₄	204.357
18	Caryophyllene	C ₁₅ H ₂₄	204.357
19	Alpha-Bergamotene	C ₁₅ H ₂₄	204.3511
20	Alpha-Humulene	C ₁₅ H ₂₄	204.357
21	Beta-Bisabolene	C ₁₅ H ₂₄	204.357
22	Delta-Cadinene	C ₁₅ H ₂₄	204.357
23	Squalene	C ₃₀ H ₅₀	410.73

CONCLUSION

Mostly seeds of *Trachyspermum ammi* are used when compared to leaves. The GC-MS analysis of *Trachyspermum ammi* leaves oil was done and found various phytoconstituents, which were identified as biomarkers that are matched with NIST library of IICT. The detection of various components in unknown sample was done by employing GC-MS technique. The result of the

study gives a platform for use of Ajwain leaves as an herbal alternative for several diseases due to their phytoconstituents.

Acknowledgement

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