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A Study on Molecular Identification of Bacteria from *Anisomeles malabarica* (L.) R. Br. Ex Sims. and its Secondary Metabolites

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Abstract

Anisomeles malabarica, an aromatic plant is widely known for its anti-diabetic properties. The current study was carried out to screen for the presence of endophytic bacteria in the leaves of *Anisomeles malabarica*. One bacterial strain was isolated from the tissues and identified as *Bacillus cereus* using 16S rRNA sequencing. Further investigation was done to explore the contribution of the endophytic bacteria in the metabolite content of the *A. malabarica* leaves. The bacterial metabolites were extracted using chloroform and carbon tetrachloride. The compounds were identified using Gas chromatography–mass spectrometry analysis. The analysis revealed the presence of some really significant metabolites like D-Limonene, Eucalyptol, terpineol, neral, cymene, Isobornyl acetate, 2,4-Di-tert-butylphenol, Dibutyl phthalate, careen etc., which are of great value. This study confirms the presence of endophytic bacteria in the *A. malabarica* leaves and its potential role in the metabolite profile of the leaves. This report is the first to isolate and identify a bacterial strain inhabiting the leaf tissues of *A.malabarica.* the bacterial isolate from plants could be a potential source of bioactive compounds.

***Key words*:** *Anisomeles malabarica, Bacillus cereus,* 16S rRNA, GC-MS analysis, Secondary metabolites

Anisomeles malabarica is a shrub that commonly inhabits the Southern part of India. The leaves have valuable medicinal properties. It is known to cure rheumatic arthritis; epilepsy and it shows analgesic activity [1]. Traditionally the leaf decoction is used to cure fever. [2] studied the water extract of *Anisomeles malabarica* leaves and stem, and reported that the analgesic activity of the plant is due to the inhibition of COX-1, which there by affects the synthesis of prostaglandins. [3] demonstrated the antiepileptic activity of the crude flavonoid fraction of its leaves. The phytochemical study on the crude extract of *Anisomeles malabarica* revealed the presence of many therapeutic active compounds belonging to flavonoid, tannin, terpenoid, and phenol group. [4-5] reported significant anti-inflammatory, anti-platelet and anti-arthritic activities demonstrated by the leaf extracts of *Anisomeles malabarica.*

Plant species playing host to a number of endophytic microorganisms have highly contributed to the ecosystem by increasing the biodiversity. The endophytes do not cause any disease to the host and they share a symbiotic relationship. Fungal species are the most frequently occurring endophytes followed by bacteria. Certain fungal species were observed to be specific to a particular plant. This phenomenon has not yet been reported in case of bacterial endophytes [6]. The success of endophyte colonization depends on a number of factors like environmental conditions, plant genotype, tissue type, microbial taxon, etc. [7]. Endophytes are known to spread systemically inside the host and colonize it [8]. Compared the bacterial endophytic isolates and rhizoplane isolates. They reported that the endophytic community is a subset of the rhizoplane community. The genus present in rhizoplane were also found to be in endophytic community and vice versa. From this observation they suggested that bacteria move from root exterior to the interior. In some cases, colonization has been observed in intercellular spaces and xylem vessels, through which they spread to other parts of the plant [9]. Bacteria have been observed colonizing various parts of the plant, including intercellular spaces and xylem vessels. These colonization sites provide pathways for bacteria to spread to other parts, facilitating their movement and distribution within the host.

Bacteria inhabiting within the plant cells have previously been reported to produce a number of valuable secondary metabolites [10]. Apart from promoting the plant growth, endophytic bacteria produce rare bioactive compounds which have significant pharmaceutical properties [11]. Isolated endophytic bacteria from several hosts and reported their potential to control nematode. Steroids were found to be the major secondary metabolites produced by the bacteria. In another study, endophytes isolated from medicinal plant were able to exhibit antibacterial activity against pathogens. The metabolite profiling revealed the presence of diisooctyl phthalate and [1-2], [4] oxadiazole, 5-benzyl-3 [12]. Camptothecine, an alkaloid with potent anticancer properties was found to be produced by endophytic bacteria isolated from *Miquelia dentate* [13]. It is to be believed that the metabolites from endophytic bacteria identified so far is just a small fraction of the existing rich metabolic repertoire. The research carried

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out and current knowledge about the endophyte metabolites is very limited.

MATERIALS AND METHODS

Collection of plant materials

The Fresh and healthy leaves of *A. malabarica* were collected from Chengattur Village, Chengalpattu District, (Latitude: 12.5268°N and Longitude: 80.0088°E) Tamil Nadu, India. during the month of January and February in a sterile bag and kept at cold and dry conditions for further use. The plant was authenticated by Botanical Survey of India (BSI) Tamil Nadu Agricultural University (TNAU) Coimbatore. (Ref no: BSI/SRC/5/23/2023/Tech.-591 date:14th Aug 2023).

Surface sterilization

The fresh leaves were washed thoroughly under running water and cut into small bits. Further steps were carried out inside the laminar air flow chamber. The surface of the leaf bits was sterilized by dipping in 75% ethanol for 5 minutes followed by sodium hypochlorite (2%) for 4 minutes [14]. The tissue was rinsed thoroughly in sterile distilled water for three times and the excess water was drained off.

Isolation of endophytic bacteria

Nutrient agar media was used throughout the experiment for isolation and maintenance of the cultures. Autoclaved Nutrient agar media was poured into sterile Petri dishes inside the laminar air flow chamber and allowed to solidify. The surface sterilized leaves were placed on the media using sterile forceps and the Petri dish was sealed using parafilm. The plates were incubated at 25±2°C and observed for 48 hours. Morphologically different bacterial colonies were repeatedly streaked in order to get bacterial isolates. All selected isolates were subculture nutrient agar slants for maintenance at 4°C and in nutrient broth for extraction.

Molecular identification of bacterial isolate

The isolated endophytic bacterial strain was identified using the 16S rRNA sequencing method. The sequencing analysis was carried out using NucleoSpin® Tissue Kit (Macherey-Nagel) following manufacturer's instructions. The 16s rRNA was amplified by Polymerase chain reaction (PCR) technique using PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems). The primers used are given in (Table 1). The PCR product purity was checked in 1.2% agarose gel prepared using 0.5X TBE buffer containing 0.5 µg/ml ethidium bromide. After electrophoresis, 5µLof PCR product was mixed with 0.5µl of ExoSAP-IT and incubated at 37ºC for 15 minutes followed by enzyme inactivation at 85ºC for 5 minutes. The product was sequenced in ABI 3500 DNA Analyzer (Applied Biosystems) using Sanger DNA sequencing method. The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1. BLAST search was done using the National Centre for Biotechnology Information (NCBI) database.

Table 1 Details of primers used

Target	Primer name	Direction	Sequence (5' → 3')
16S rRNA	16S-RS-F	Forward	AGGCCTAACACATGCAAGTC
	16S-RS-R	Reverse	GGGCGGWGTGTACAAGGC

Sequence analysis

The sequence quality was checked using sequence scanner software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1 [26].

Extraction of bacterial metabolites

The secondary metabolites from the endophyte were extracted by inoculating 200 µL of bacterial suspension into 250 ml of sterile nutrient broth media. The culture was incubated at 37 °C for 5 days in Orbitolshaker, at 150 rpm. After the incubation period, the culture broth was filtered and filtrates were added with an equal volume of 1:1. (a) Chloroform (250 ml) and (b) Carbon tetrachloride individually. The mix was left overnight at 4 °C. Using a separating funnel, the solvent layer containing the extracted metabolites was collected and evaporated in a rotary evaporator (90 rpm, 40 °C) to obtain the crude metabolites [15].

Analysis of secondary metabolites

The bioactive compounds present in the crude extract were analyzed using gas chromatography-mass spectrometry (GC-MS). It was performed at a flow rate of 1.0 ml per minute. The evaluation was done two times.

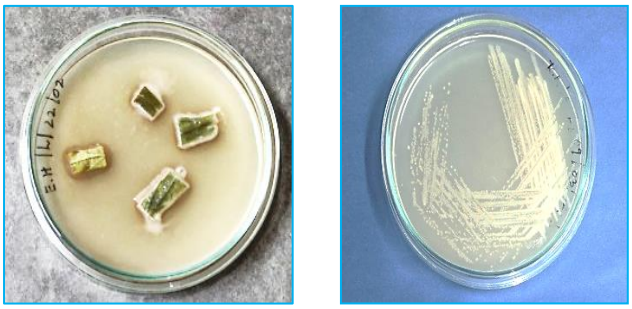
Description of components

Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. 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 and structure of the components of the test materials were confirmed.

RESULTS AND DISCUSSION

Isolation and identification of endophytic bacteria

One bacterial strain was isolated from the leaf segments of *A. malabarica* (Fig 1). 16S rRNA sequencing revealed that the endophytic bacteria belong to the genus *Bacillus*. BLAST search revealed that the isolate is most related to *Bacillus cereus* with 99.6% of sequence identity. The 16S rRNA Gene Sequence (Fig 2). The sequence is deposited to the National Centre for Biotechnology Information (NCBI) Gene bank with Accession No: SUB13584224.



(A) Endophyte culture initiated from leaf bits

(B) pure culture from isolate

Fig 1 Isolation of endophytic bacteria

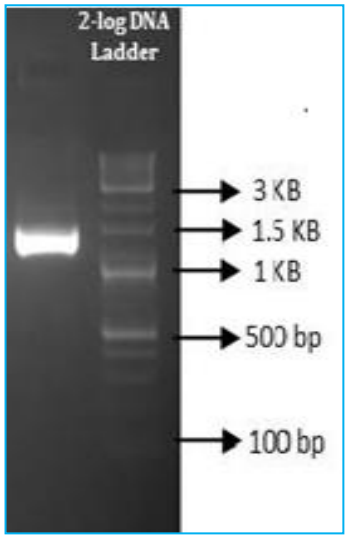


Fig 16S rRNA Gene amplification of bacteria

So far there is only report on isolation of endophytic fungi from *A. malabarica* [16]. No studies have been reported on the endophytic bacteria of *A. malabarica*. Since the knowledge on metabolites profile of bacteria is far from saturated, they are of special interest. The leaves of *A. malabarica* have shown significant biological activities such as antidiabetic, antiepileptic, antimicrobial etc., owing to its metabolites [17-18]. The endophytes dwelling in the tissues are also a great source of such valuable metabolites. Hence, in the present study endophytic bacteria was isolated to investigate its metabolic repertoire.

Secondary metabolite profiling

GC-MS chromatogram revealed a total of 34 compounds in Carbon tetrachloride extract (Fig 3) and 24 compounds in chloroform extract (Fig 4). The details are given in (Table 2-3). The chloroform extract was found to have some industrially and pharmaceutically important compounds like D-Limonene, Eucalyptol, terpineol, neral, cymene, carene etc.

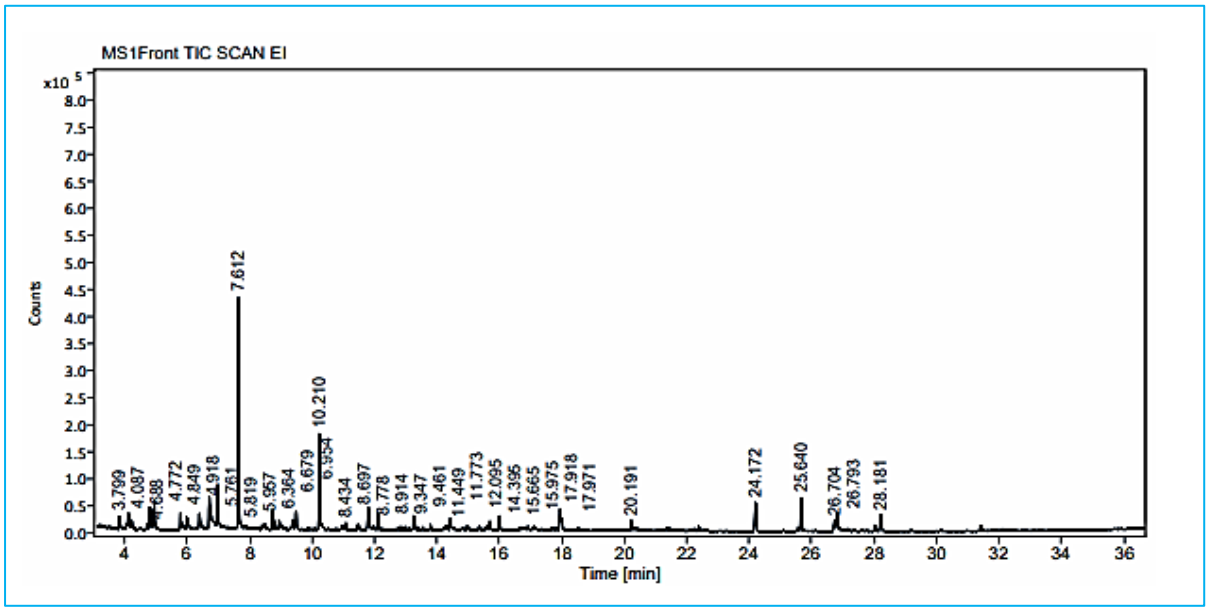


Fig 2 GC-MS chromatogram of carbon tetrachloride extract

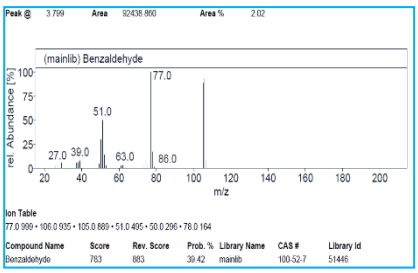


Fig 2.1 Benzaldehyde

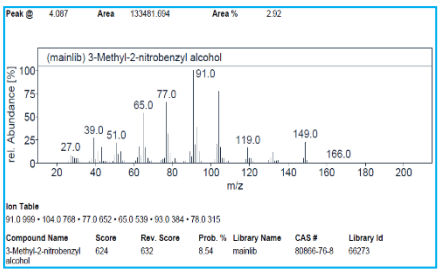


Fig 2.2 3-Methyl-2-nitrobenzyl alcohol

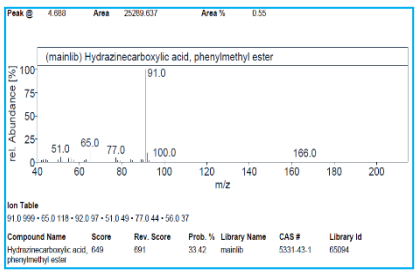


Fig 2.3 Hydrazinecarboxylic acid, phenylmethyl ester

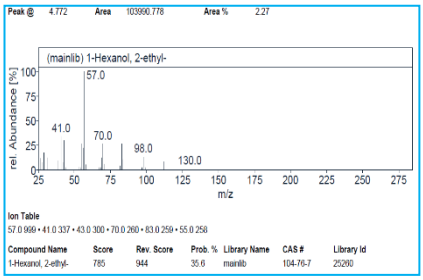


Fig 2.4 1-Hexanol, 2-ethyl-

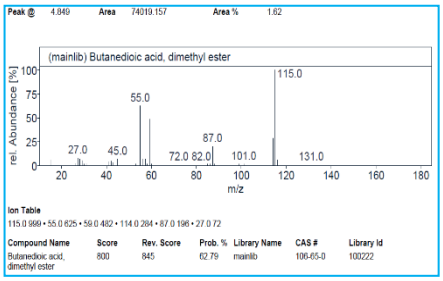


Fig 2.5 2-Bromopropionic acid, 2-ethylhexyl ester

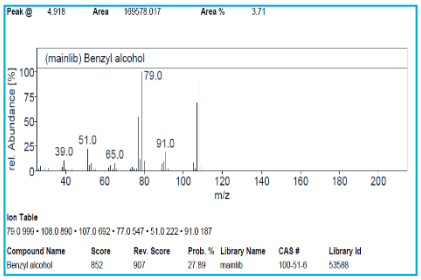


Fig 2.6 Benzyl alcohol

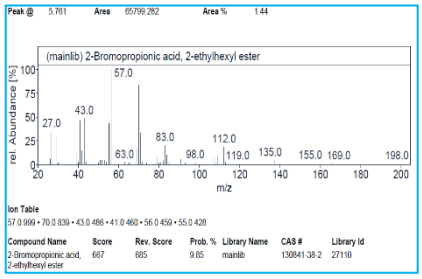


Fig 2.7 2-Bromopropionic acid, 2-ethylhexyl ester

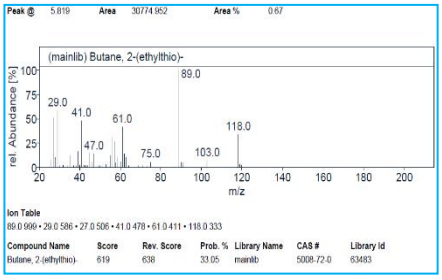


Fig 2.8 Butane, 2-(ethylthio)-

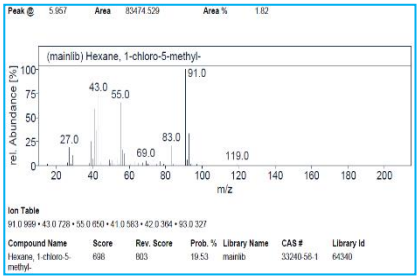


Fig 2.9 Hexane, 1-chloro-5-methyl-

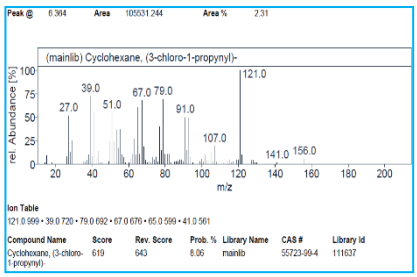


Fig 2.10 Cyclohexane, (3-chloro-1-propynyl)-

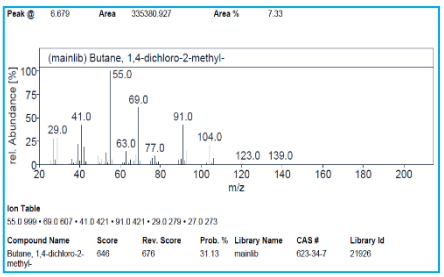


Fig 2.11 Butane, 1,4-dichloro-2-methyl-

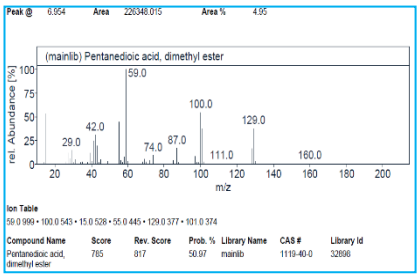


Fig 2.12 Pentanedioic acid, dimethyl ester

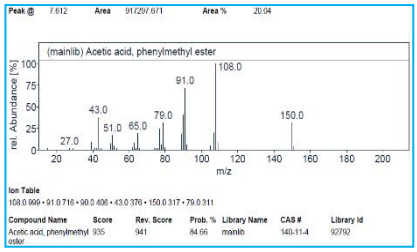


Fig 2.13 Acetic acid, phenylmethyl ester

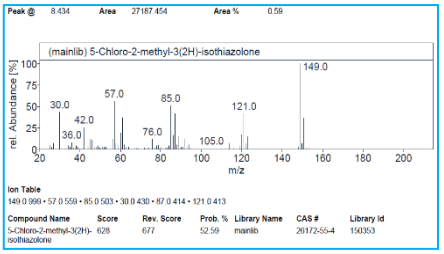


Fig 2.14 5-Chloro-2-methyl-3(2H)-isothiazolone

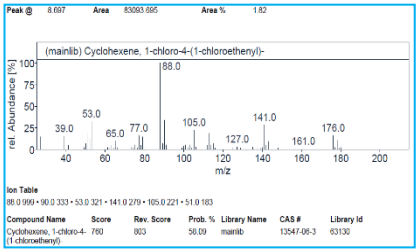


Fig 2.15 Cyclohexene, 1-chloro-4-(1-chloroethenyl)-

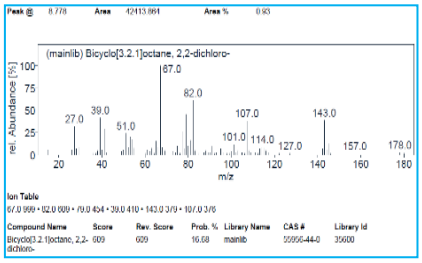


Fig 2.16 Bicyclo[3.2.1]octane, 2,2-dichloro-

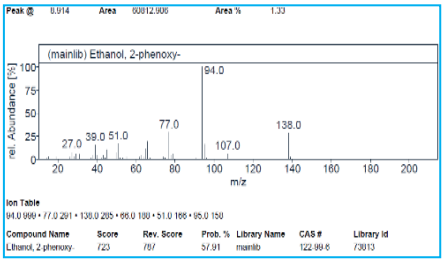


Fig 2.17 Ethanol, 2-phenoxy

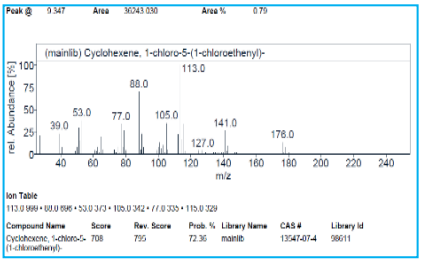


Fig 2.18 Cyclohexene, 1-chloro-5-(1-chloroethenyl)-

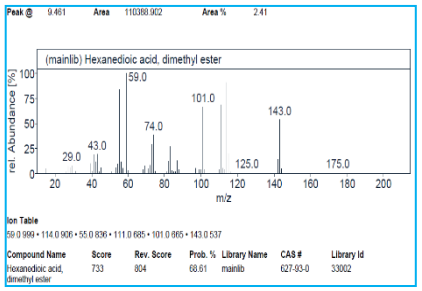


Fig 2.19 Hexanedioic acid, dimethyl ester

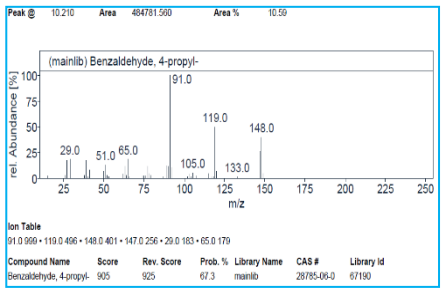


Fig 2.20 Benzaldehyde, 4-propyl-

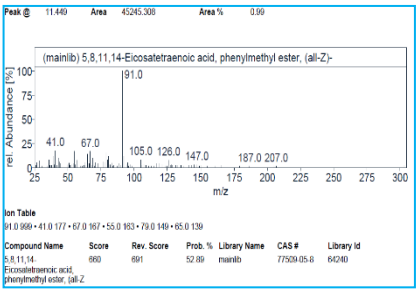


Fig 2.21 5,8,11,14-Eicosatetraenoic acid,

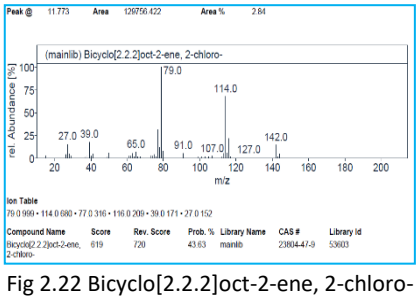


Fig 2.22 Bicyclo[2.2.2]oct-2-ene, 2-chloro-phenylmethyl ester, (all-Z)-

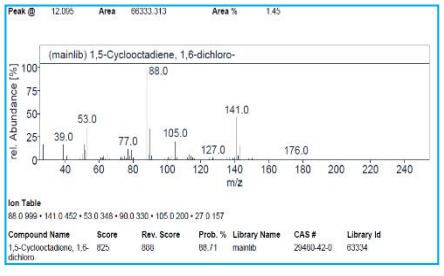


Fig 2.23 1,5-Cyclooctadiene, 1,6-dichloro-

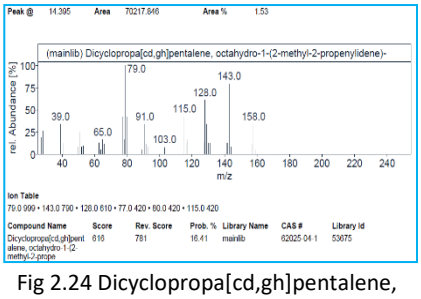


Fig 2.24 Dicyclopropa[cd,gh]pentalene, octahydro-1-(2-methyl-2-propenylidene)-

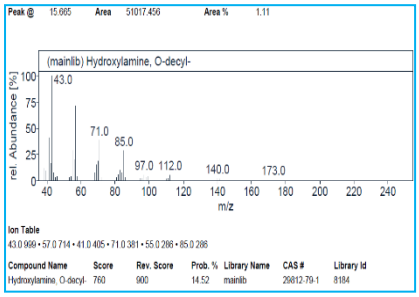


Fig 2.25 Hydroxylamine, O-decyl-

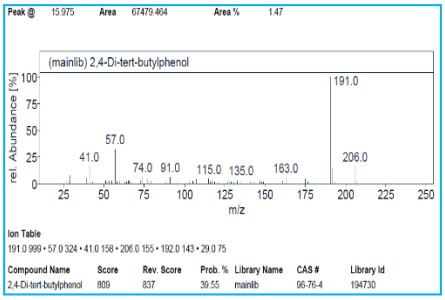


Fig 2.26 2,4-Di-tert-butylphenol

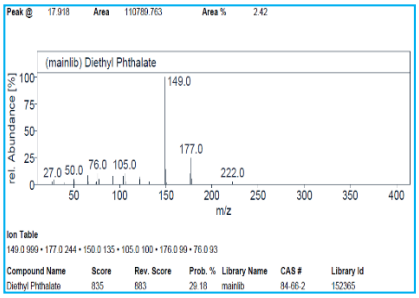


Fig 2.27 Diethyl Phthalate

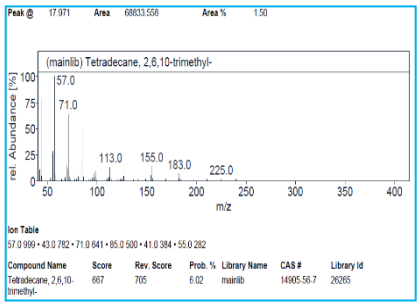


Fig 2.28 Tetradecane, 2,6,10-trimethyl-

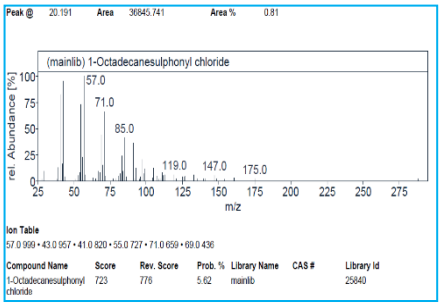


Fig 2.29 1-Octadecanesulphonyl chloride

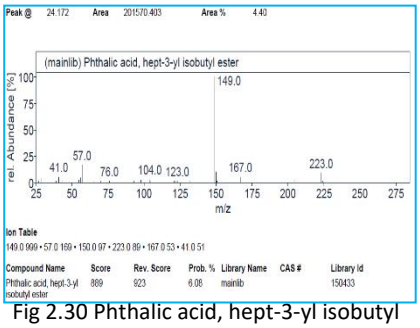


Fig 2.30 Phthalic acid, hept-3-yl isobutyl ester

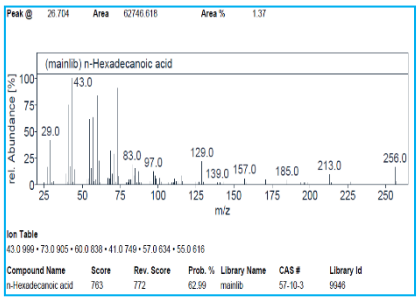


Fig 2.31 n-Hexadecanoic acid

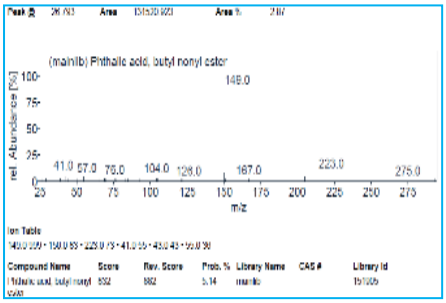


Fig 2.32 Phthalic acid, butyl nonyl ester

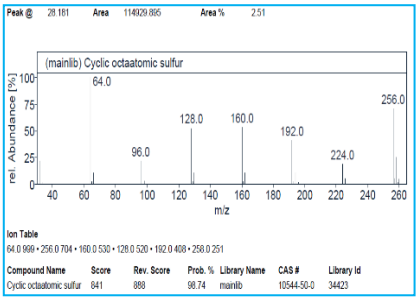


Fig 2.33 Cyclic octaatomic sulphur

Table 2 Major compounds profile of carbon tetrachloride extract

S. No.	Retention time	Name of the compounds	Molecular formula	Molecular weight	Peak area
1	3.799	Benzaldehyde	C_7H_6O	106.12	2.02
2	4.087	3-Methyl-2-nitrobenzyl alcohol	$C_8H_9NO_3$	167.16	2.92
3	4.688	Hydrazinecarboxylic acid, phenylmethyl ester	$C_8H_{10}N_2O_2$	166.17	0.55
4	4.772	1-Hexanol, 2-ethyl-	$C_8H_{18}O$	130.22	2.27
5	4.849	Butanedioic acid, dimethyl ester	$C_6H_{10}O_4$	146.16	1.62
6	4.918	Benzyl alcohol	C_7H_8O	108.14	3.71
7	5.761	2-Bromopropionic acid, 2-ethylhexyl ester	$C_{11}H_{21}BrO_2$	265.19	1.44
8	5.819	Butane, 2-(ethylthio)-	$C_6H_{14}S$	118.24	0.67
9	5.957	Hexane, 1-chloro-5-methyl-	$C_7H_{15}CL$	134.65	1.82
10	6.364	Cyclohexane, (3-chloro-1-propynyl)-	$C_9H_{13}Cl$	156.65	2.31
11	6.679	Butane, 1,4-dichloro-2-methyl-	$C_5H_{10}CL_2$	141.03	7.33
12	6.954	Pentanedioic acid, dimethyl ester	$C_7H_{12}O_4$	160.16	4.95
13	7.612	Acetic acid, phenylmethyl ester	$C_9H_{10}O_2$	150.17	20.04
14	8.434	5-Chloro-2-methyl-3(2H)-isothiazolone	C_4H_4CINOS	149.60	0.59
15	8.697	Cyclohexene, 1-chloro-4-(1-chloroethenyl)-	$C_8H_{10}CL_2$	177.07	1.82
16	8.778	Bicyclo[3.2.1]octane, 2,2-dichloro-	$C_8H_{14}O_2$	142.19	0.93
17	8.914	Ethanol, 2-phenoxy	$C_8H_{10}O_2$	138.16	1.33
18	9.347	Cyclohexene, 1-chloro-5-(1-chloroethenyl)-	$C_8H_{10}CL_2$	177.07	0.79
19	9.461	Hexanedioic acid, dimethyl ester	$C_8H_{14}O_4$	174.19	2.41
20	10.210	Benzaldehyde, 4-propyl-	$C_{10}H_{14}O$	148.20	10.59
21	11.449	5,8,11,14-Eicosatetraenoic acid, phenylmethyl ester, (all-Z)-	$C_{21}H_{34}O_2$	318.49	0.99
22	11.773	Bicyclo[2.2.2]oct-2-ene, 2-chloro-	C_8H_{12}	108.18	2.84
23	12.095	1,5-Cyclooctadiene, 1,6-dichloro-	$C_8H_{10}CL_2$	177.07	1.45
24	14.395	Dicyclopropa[cd,gh]pentalene, octahydro-1-(2-methyl-2-propenylidene)-	$C_{12}H_{14}$	158.24	1.53
25	15.665	Hydroxylamine, O-decyl-	$C_{10}H_{23}NO$	173.30	1.11
26	15.975	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.32	1.47
27	17.918	Diethyl Phthalate	$C_{12}H_{14}O_4$	222.24	2.42

28	17.971	Tetradecane, 2,6,10-trimethyl-	$C_{17}H_{36}$	240.46	1.50
29	20.191	1-Octadecanesulphonyl chloride	$C_{18}H_{37}CLO_2S$	353.0	0.81
30	24.172	Phthalic acid, hept-3-yl isobutyl ester	$C_{19}H_{28}O_4$	320.4	4.40
31	25.640	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276.4	4.61
32	26.704	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4	1.37
33	26.793	Phthalic acid, butyl nonyl ester	$C_{23}H_{36}O_4$	376.52	2.87
34	28.181	Cyclic octaatomic sulphur	S_8	256.52	2.51

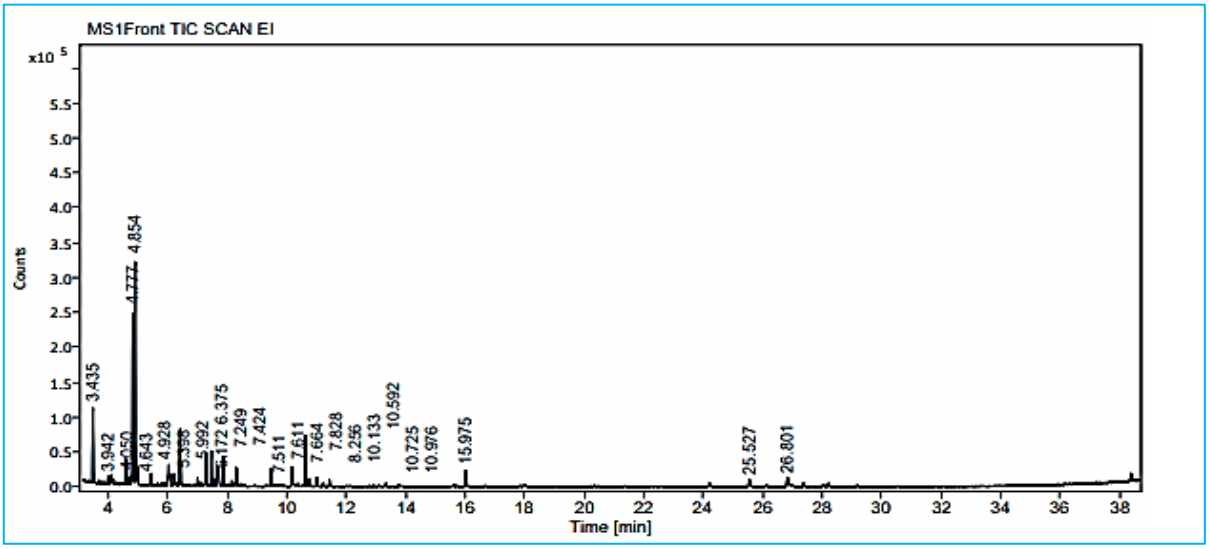


Fig 3 GC-MS chromatogram of chloroform extract

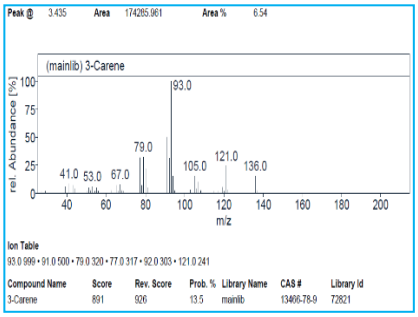


Fig 3.1 3-Carene

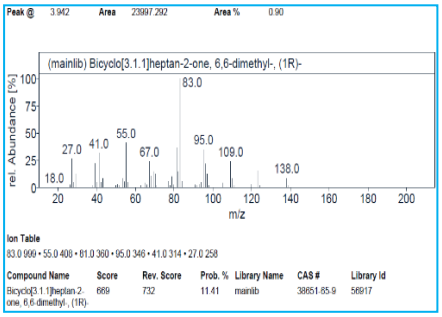


Fig 3.2 Bicyclo[3.1.1]heptan-2-one, 6,6-dimethyl-, (1R)-

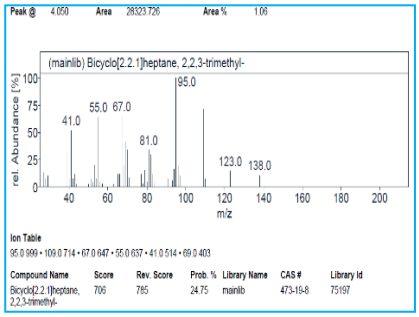


Fig 3.3 Bicyclo[2.2.1]heptane,2,2,3-trimethyl-

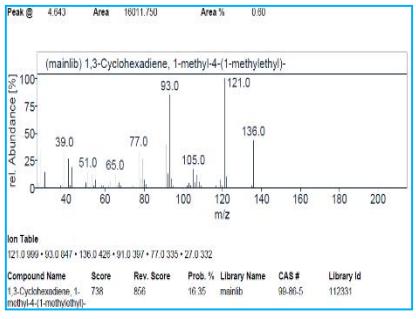


Fig 3.4 1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-

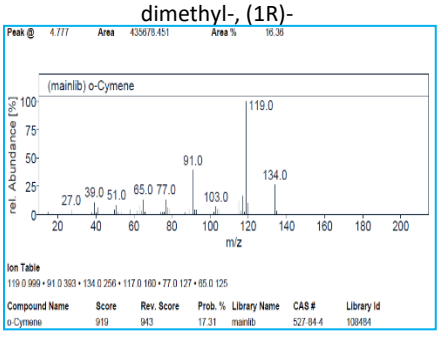


Fig 3.5 o-Cymene

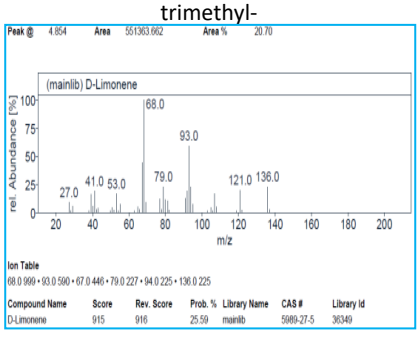


Fig 3.6 D-Limonene

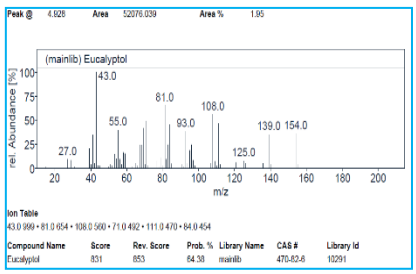


Fig 3.7 Eucalyptol

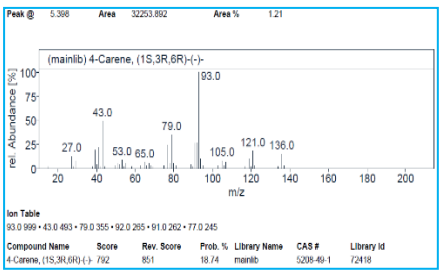


Fig 3.8 4-Carene, (1S,3R,6R)-(-)-

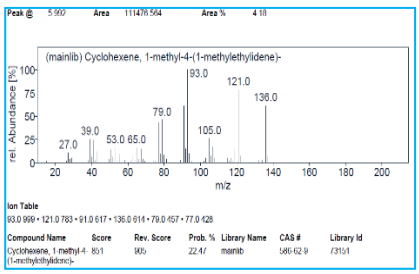


Fig 3.9 Cyclohexene, 1-methyl-4-(1-methylethylidene)-

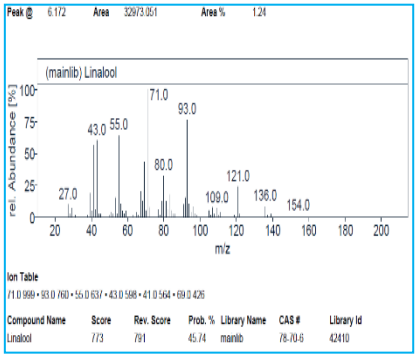


Fig 3.10 Linalool

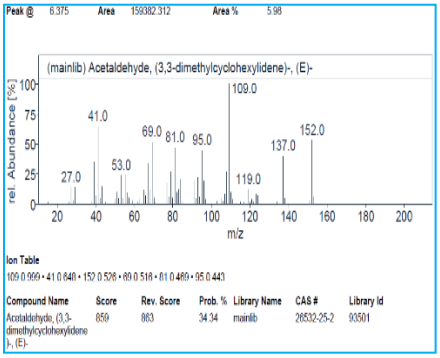


Fig 3.11 Acetaldehyde, (3,3-dimethylcyclohexylidene)-,(E)-

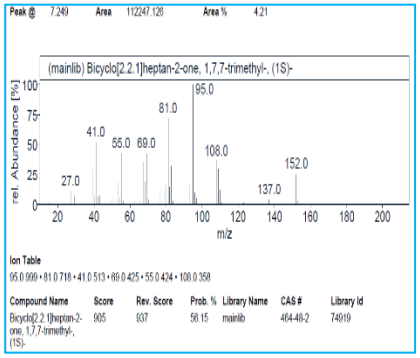


Fig 3.12 Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-

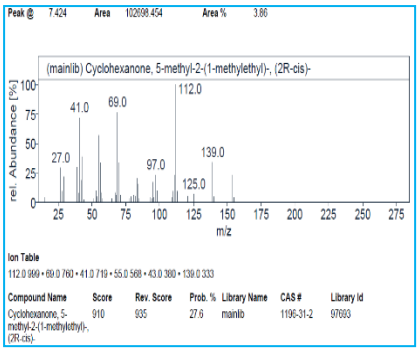


Fig 3.13 Cyclohexanone, 5-methyl-2-(1-methylethyl)-, (2R-cis)-

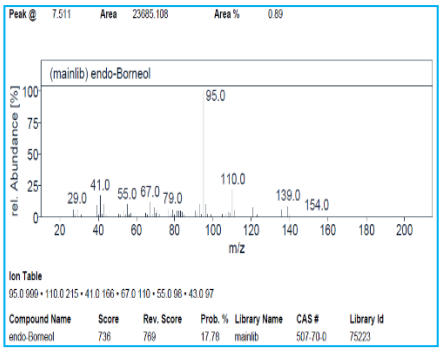


Fig 3.14 Endo-Borneol

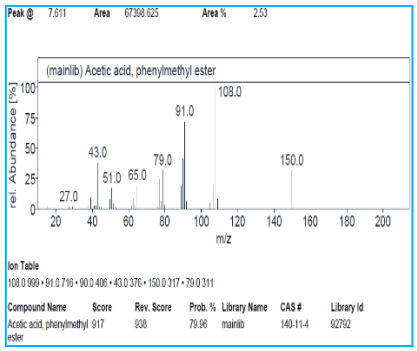


Fig 3.15 Acetic acid, phenylmethyl ester

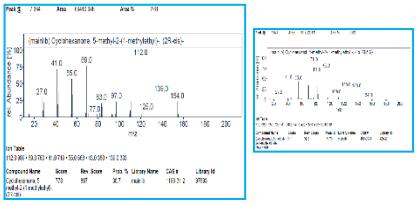


Fig 3.16 Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1α,2β,5β)-

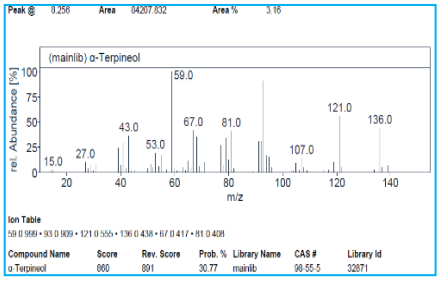


Fig 3.17 α-Terpineol

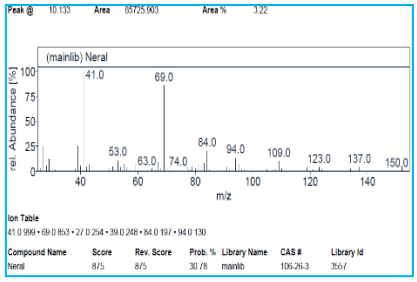


Fig 3.18 Neral

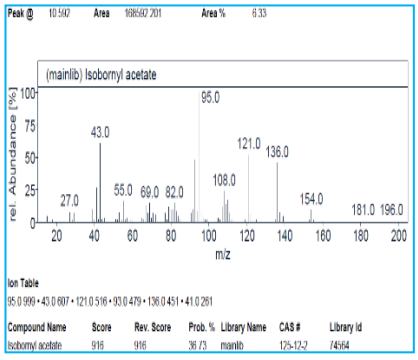


Fig 3.19 Isobornyl acetate

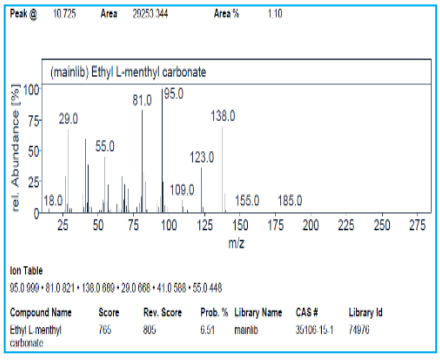


Fig 3.20 Ethyl L-menthyl carbonate

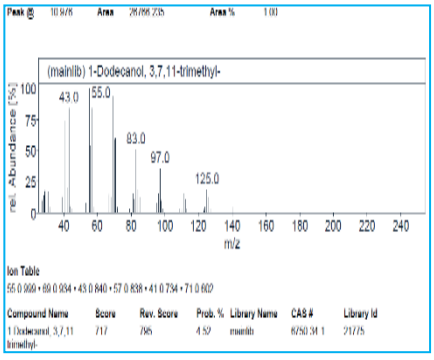


Fig 3.21 1-Dodecanol, 3,7,11-trimethyl-

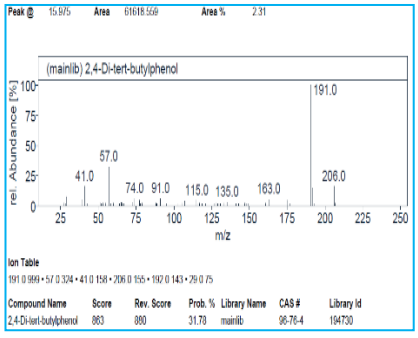


Fig 3.22 2,4-Di-tert-butylphenol

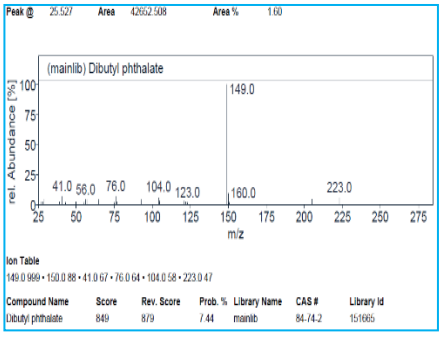


Fig 3.23 Dibutyl phthalate

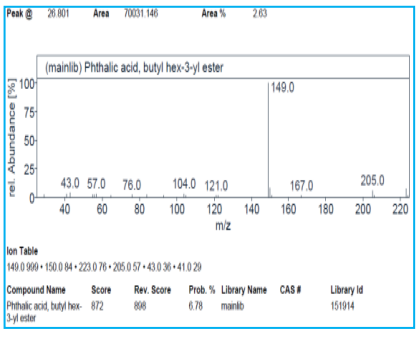


Fig 3.24 Phthalic acid, butyl hex-3-yl ester

Table 2 Major compounds profile of chloroform extract

S. No.	Retention time	Name of the compounds	Molecular formula	Molecular weight	Peak area
1	3.435	3-Carene	$C_{10}H_{16}$	136.23	6.54
2	3.942	Bicyclo[3.1.1]heptan-2-one, 6,6-dimethyl-, (1R)-	$C_9H_{14}O$	138.20	0.90
3	4.050	Bicyclo[2.2.1]heptane, 2,2,3-trimethyl-	$C_{10}H_{18}$	138.24	1.06
4	4.643	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	$C_{10}H_{16}$	136.23	0.60
5	4.777	o-Cymene	$C_{10}H_{14}$	134.22	16.36
6	4.854	D-Limonene	$C_{10}H_{16}$	136.23	20.70
7	4.928	Eucalyptol	$C_{10}H_{18}O$	154.24	1.95
8	5.398	4-Carene, (1S,3R,6R)-(-)-	$C_{10}H_{16}$	136.23	1.21
9	5.992	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	$C_{10}H_{16}$	136.23	4.18
10	6.172	Linalool	$C_{10}H_{18}O$	154.25	1.24
11	6.375	Acetaldehyde, (3,3-dimethylcyclohexylidene)-, (E)-	$C_{10}H_{16}O$	152.23	5.98
12	7.249	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	$C_{10}H_{16}O$	152.23	4.21
13	7.424	Cyclohexanone, 5-methyl-2-(1-methylethyl)-, (2R-cis)-	$C_{10}H_{18}O$	154.24	3.86
14	7.511	endo-Borneol	$C_{10}H_{18}O$	154.25	0.89
15	7.611	Acetic acid, phenylmethyl ester	$C_9H_{10}O_2$	150.17	2.53
16	7.828	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1α,2β,5β)-	$C_{10}H_{20}O$	156.26	3.82
17	8.256	α-Terpineol	$C_{10}H_{18}O$	154.25	3.16
18	10.133	Neral	$C_{10}H_{16}O$	152.23	3.22
19	10.592	Isobornyl acetate	$C_{12}H_{20}O_2$	196.29	6.33
20	10.725	Ethyl L-menthyl carbonate	$C_{13}H_{24}O_3$	228.32	1.10
21	10.976	1-Dodecanol, 3,7,11-trimethyl-	$C_{15}H_{32}O$	228.41	1.00
22	15.975	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.32	2.31
23	25.527	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.34	1.60
24	26.801	Phthalic acid, butyl hex-3-yl ester	$C_{18}H_{26}O_4$	306.39	2.63

Even though Carbon tetrachloride solvent was comparatively found to extract more number of compounds, chloroform was able to extract out some really valuable secondary metabolites with wide application. The compounds that were found to be produced by the endophytic bacteria have previously been reported to have significant bioactivities. α-Terpineol which was found in the chloroform extract, is known for anticancer, antihypertensive, antibronchitis, antioxidant and antiulcer activities [19]. D-Limonene, a terpenoid is widely being investigated for its anticancer property. It is known for its chemotherapeutic and chemo preventive effects [20] demonstrated the capacity of limonene to get distributed in human breast tissues thereby reducing the expression of breast tumor cyclin D1, which causes cell cycle arrest and reduced cell proliferation. p-Cymene, another major constituent of chloroform extract was found to prevent the proliferation of liver carcinoma, gastric carcinoma and nasopharyngeal carcinoma. Apart from that it exhibited cytotoxicity against human carcinoma cell lines [21]. Traces of Eucalyptol was also found to be produced by the endophyte. Some of the volatile compounds found in the Carbon tetrachloride extract are known to exhibit antimicrobial activity.

The components of medicinal plants reveal enormous pharmaceutical applications and unidentified components is plenty in the biological resources. The developing countries need of the novel pharmaceutically important components is essential for the resistance diseases. In spite of existing drugs there is essential from combinational or biological resources. There are the studies of metabolites of bacterial sources are restricted from the bacteria from plant origin. The present study is focused in the isolation of bacteria from *Anisomeles malabarica* exhibits the presence of *Bacillus cereus* based on the PCR amplification of bacterial 16S rRNA. Similarly isolated the *Bacillus cereus* from the leaf of *Garcinia xanthochymus* and proved by 16S rRNA sequencing analysis of which 99% similarity it is proved that culture independent methods like PCR amplification of bacteria 16 rRNA is highly significance for the identification for bacterial species [22].

Bacillus cereus has previously been reported as an endophyte in many plant hosts. *B. Cereus* isolated from mustard was found to show chitinolytic activity [23]. The strain isolated from *Sophora alopecuroides* root nodule sand sunflower roots was found to exhibit plant growth promoting traits [24-25]. Considering the significance of the bioactive compounds reported in the current study we propose further research on compound purification, identification and exploring their bioactivities. This could add to the existing knowledge about endophytic bacteria.

Acknowledgment

I am honored to thank Secretary and Principal, Ramakrishna Mission Vivekananda College (Autonomous), Chennai. We would like to thank Rajiv Gandhi Centre for Biotechnology, Regional Facility for DNA Fingerprinting, Thiruvananthapuram, Kerala, India for 16s RNA sequencing and I specially thank Sophisticated Analytical Instrument Facility (SAIF) Indian Institute of Technology, Chennai, India, to carry out GC-MS studies.

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