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COMPARATIVE ACCOUNT ON GC-MS ANALYSIS OF *MENTHA ARVENSIS* L. "CORN MINT" FROM THREE DIFFERENT LOCATIONS OF NORTH INDIA

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ABSTRACT

The essential oil analysis of Mentha arvensis L. (Corn Mint) has been done for the first time from three locations of North India. The extraction yields for the essential oils of three locations of M. arvensis were: 0.38% for sample M-1 collected from Fatehpur (415m), 0.31% for sample M-2 from Dhameta (435m) and 0.36% for sample MP from Patiala (250m). The oils were analyzed by GC-MS, the components of oil were identified by comparing their retention indices and mass spectra fragmentation patterns with those stored on the MS-computer library and also from the published literatures. The major constituents reported from essential oils of M. arvensis were: L-Menthone, Menthol, Isomenthone, Eucalyptol, Piperitone oxide, Carvone, dl-Limonene, trans-Dihydrocarvone, Germacrene-D, etc. from all three samples collected from Punjab and Himachal Pradesh of Northern India.

KEY WORDS: *Mentha arvensis L., Corn Mint, Essential oil, GC-MS, North India.*

INTRODUCTION

Mentha arvensis L. (Lamiaceae), commonly known as cornmint, menthol mint or Japanese mint was introduced into India in 1952 from Japan. Cornmint plants consist of shoots, having over ground main stems with big leaves and small flowers, stolons, with crawling succulent stems and underground rhizomes. Essential oils obtained from natural sources are important raw materials in the perfumes and flavour industry. The natural origin of some components lends them great importance as premium materials in applications such as food grade flavours. Some compounds of *M. arvensis* L. were widely used as cooling compound in mint flavours, fruit flavours, oral care products, confections and beverages. [1]

In Indian folk medicine numerous plant products are used in the regulation of human fertility. Amongst these, the leaves of *Mentha arvensis* L. (Lamiaceae), the common edible aromatic herb, has been described to possess various medicinal properties including an anti-fertility effect. [2, 3] *Mentha arvensis* L. is commercially cultivated in tropical and subtropical climates. The oil and a by-product, menthol and dementholized oil (DMO), respectively of this plant have the highest share in the global mint trades. [4] *Mentha arvensis* L. is cultivated in many parts of the world for the production of menthol from its essential oil which is used in pharmaceutical, perfumery and food industries. Besides China and United States of America, India is a major producer of mint with an annual production of about 5000 tonnes of essential oil. Present estimates indicate that the crop is cultivated in approximately 100000 hectares in India, with the estimated production of approximately 15000 tonnes of volatile oil during 1997. The bulk of this production comes from Badaun, Barelly, Bilaspur, Moradabad, Nainital, Rampur (tarai tract), Barabanki and Luckhnow (Indo-Gangetic plains) districts of the State of Uttar Pradesh (North India). The rest of the production

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originates in the state of Punjab and Himachal Pradesh (North-West India). Field experiments conducted under the semi-arid tropical climatic conditions of Andhra Pradesh State (South India) showed that the crop can be successfully grown in this climate also. [5] Large efforts of genetic improvement in menthol mint made via proper exploitation of the advantages of sexual and asexual (vegetative) means of propagation. [6-10] Therefore, with the establishment of superior cultivars, India is emerging as the largest producer (70%) of menthol mint oil in the world. [4]

Volatile oil is extracted mainly from the shoots, the composition of which has been studied in detail. [11, 12] Several GC-MS reports were given by workers on *M. arvensis* L. [13-15] But, there is not even a single comparative report available about the compositions of essential oils of *M. arvensis* L. from these three locations of Northern India.

Therefore, as a part of our investigation on chemical evaluations of aromatic and medicinal plants, the aim of this work is to provide information about the comparative accounts about the constituents of essential oils obtained from *M. arvensis* L. populations, collected from three different locations of Punjab and Himachal Pradesh.

EXPERIMENTAL

Plant material

Fresh leaves of *Mentha arvensis* L. were collected from three different places of Fatehpur M-1 (415m), Dhameta M-2 (435m) of Himachal Pradesh and Patiala MP (250m) of Punjab from Northern India, during the month of April, 2008 (Table 1). The specimens were deposited in the Herbarium, Department of Botany, Punjabi University, Patiala (Punjab) India.

Oil distillation

Five hundred grams fresh sample of leaves from three places were separated and grounded, then immersed in water in a round bottom flask and hydrodistilled for 4h in a full glass Clevenger-type apparatus as recommended by British Pharmacopoeia giving yellowish oils. The essential oil was dried over anhydrous sodium sulphate (Merck) until the last traces of water were removed and then stored in a dark glass bottle at 4 ºC prior to GC-MS analysis. [16]

Gas chromatography-Mass-spectrometry

GC-MS (70ev) data were measured on GC-MS (QP 2010 series Shimadzu, Tokyo, Japan) equipped with AOC 20i autosampler and BP-20 capillary column (SGC International Ringwood, Australia) of 30m length, 0.25mm i.d. and 0.25µm film thickness. Temperature was

Table 1. Collection details and essential oils yield of *M. arvensis* L. from four locations of Northern India.

S. No.	Species name	Sample codes	Place of collection	Altitude of study area (m)	Month & year of collection	Oil yield (%)
1.	*Mentha arvensis* L.	M-1	Fatehpur (H.P.)	415	April, 2008.	0.38
2.	*Mentha arvensis* L.	M-2	Dhameta (H.P.)	435	April, 2008.	0.31
3.	*Mentha arvensis* L.	MP	Patiala (Pb.)	250	April, 2008.	0.36

H.P. : Himachal Pradesh; Pb.: Punjab; m: meter; %: Percentage

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programmed from 70-220 ºC at a rate of 4 ºC/min, held isothermally at 70 ºC and 220 ºC for 4 and 5 min, respectively. Mass spectrometer source temperature, 200 ºC; interface temperature, 220 ºC; injector temperature, 220 ºC. Sample injection volume 2µL (diluted 5µL oil in 2mL dichloromethane, HPLC grade); split ratio, 1:50 and mass scan, 50-600 amu. Helium was used as a carrier gas with 1.1mL/min flow rate.

Identification of components

The retention index was calculated for all volatile constituents using a homologous series of *n*-alkanes. The components of oil were identified by matching their mass-spectra with those stored in the computer library such as Wiley, New York mass spectral (MS) library and their retention indices (RI) either with authentic compounds or with published data in the literature based on retention indices of components on same phases of polar columns such as: BP-20, CW-20M, HP-20M and Supelcowax-10, etc.

Results and Discussion

The volatile oils from three locations of *M. arvensis* L. were obtained by conventional hydro distillation, which gave yellowish oils. The extraction yields for the essential oils were 0.38% (M-1), 0.31% (M-2) and 0.36% (MP) for all the three samples. By gas chromatography mass spectroscopy (GC-MS) analysis the components of the essential oil were identified. The essential oil analysis led to the identification of 21 constituents representing 92.83% for (M-1); 17 constituents out of total 40 constituents, representing 78.99% for (M-2) and 31 constituents representing 97.03% for (MP) of the compositions of oil.

The major constituents reported from essential oils of *M. arvensis* (M-1) collected from Fatehpur (415m) were: L-Menthone (29.41%); Menthol (21.33%); Isomenthone (10.80%); Eucalyptol (6.91%); neo-Menthol (4.70%); *cis*-Piperitone oxide (3.62%); Linalool (2.20%); Thymol (1.60%); dl-Limonene (1.47%); α-Phellandrene (3.20%) and along with some major constituents some minor constituents were also presented (Table 2).

Table 2. Volatile oil composition of *M. arvensis* (M-1) from Fatehpur (415m).

S. No.	RT[a]	Constituents	RI[b]	RI[c]	RA[d]
1.	3.871	dl-Limonene	---	1154	1.47
2.	4.048	Eucalyptol	---	1206	6.91
3.	4.458	α-Pinene	---	1039	0.68
4.	4.662	α-Pinene	---	1039	1.13
5.	4.787	δ-3-Carene	1201	1147	0.20
6.	5.173	α-Phellandrene	1217	1216	3.20
7.	6.782	Octyl cyclobutanecarboxylate	1283	---	0.32
8.	8.367	3-Octanol	1340	1382	1.82
9.	10.207	L-Menthone	1402	1456	29.41
10.	10.367	*cis*-Sabinene hydrate	1407	1520	0.69
11.	10.962	Isomenthone	1426	1452	3.82

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12.	12.916	Linalool	1487	1538	2.20
13.	13.134	neo-Menthol acetate	1494	---	0.29
14.	13.873	*trans*-Caryophyllene	1518	---	0.51
15.	14.210	neo-Menthol	1529	1599	4.70
16.	14.342	4-Terpineol	1533	1551	0.29
17.	15.544	Menthol	1573	1612	21.33
18.	16.292	*trans*-Anethole	1597	1809	1.62
19.	16.445	δ-Terpineol	1602	1655	0.21
20.	17.135	2-Acetylfuran	1625	---	1.36
21.	17.225	α-Terpineol	1628	1687	0.42
22.	17.387	*cis*-Piperitone oxide	1633	1700	3.62
23.	17.977	Isomenthone	1653	1452	6.98
24.	18.208	5-Isopropyl-6,7-epoxy-8-hydroxy-8-methylnon-2-one	1661	---	0.34
25.	22.751	2,6,6-Trimethyl-cyclohex-1-enecarboxylic acid	1669	---	0.41
26.	24.358	3-Methyl-3-(4-methyl-3-pentenyl)-oxiranemethanol	1875	---	0.16
27.	24.586	Caryophyllene oxide	1883	1927	0.53
28.	27.781	2,5-Dimethyl-3-hexyne-2,5-diol	2001	---	0.51

RT[a] : Retention time.
RI[b] : Retention indices according to their elution order on BP-20 polar column.
RI[c] : Actual retention indices of components on same phases of columns (BP-20, CW-20M, HP-20M and Supelcowax-10).
RA[d] : Percentage of components.
--- : RI cannot calculate

Major constituents reported from *M. arvensis* L. (M-2) collected from Dhameta (435m) were: L-Menthone (27.10%); Menthol (20.25%); Piperitone oxide (6.48%); Isoneomenthone (4.13%); Eucalyptol (3.96%); L-Linalool (1.99%); Piperitone oxide (9.89%); Thymol (1.49%) (Table 3)

In third samples of *M. arvensis* L. (MP) collected from Patiala (250m), the major constituents reported were: Carvone (60.25%); dl-Limonene (19.34%); *trans*-Dihydrocarvone (6.36%); Germacrene-D (2.37%); 1-Carveol (1.62%) and Dihydrocarveol (1.02%). Along with some major constituents, minor constituents were also reported which play a great role in flavour of the oil (Table4).

In all these populations of *M. arvensis*, the percentage of L-Menthone, Menthol and Isomenthone reported to be higher in (M-1) sample than (M-2). Whereas, these constituents were not reported in third sample (MP) collected from Patiala (250m) of Punjab, in which Carvone, dl-Limonene and *trans*-Dihydrocarvone were reported to be maximum. We have not find out huge differences among the percentage of some major constituents i.e. L-Menthone, Menthol and Isomenthone, reported in (M-1) and (M-2) samples from Himachal Pradesh. It may be due to same environmental conditions

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and near about same altitude between these two study areas. Hence, do not impart any serious effect on the percentage of some major constituents. On the other hand the environmental conditions and altitude of Patiala (Punjab) with respect to Fatehpur and Dhameta of Himachal Pradesh is different to each other. That may be one of the reasons about the differences in the percentage of some major constituents. GCMS chromatograms for three populations are given (Figures 1-3).

Several GC-MS reports were given by many workers on *M. arvensis* L. such as: Essential oil composition and chemoarrays of menthol mint (*Mentha arvensis* L. f. *piperascens* Malinvaud ex. Holmes) cultivars [13], yield and resource use optimization in late transplanted mint (*Mentha arvensis* L.) under subtropical conditions [14], Identification and quantification of L-menthyl lactate in essential oils from *Mentha arvensis* L. from India and model studies on the formation of L-menthyl lactate during essential oil production [15] and recovery of dissolved essential oils from condensate waters of basil and *Mentha arvensis* distillation [17].

But the aim of this study is to provide more information about the essential oil constituents of *M. arvensis* L. from different locations. Hence, it is the first record of essential oils compositions of *M. arvensis* L. from different regions of (Punjab and Himachal Pradesh) of North India, which were previously not described from these study areas.

Table 3. Volatile oil composition of *M. arvensis* (M-2) from Dhameta (435m).

S. No.	RT[a]	Constituents	RI[b]	RI[c]	RA[d]
1.	3.874	dl-Limonene	---	1154	0.58
2.	4.047	Eucalyptol	---	1206	3.96
3.	4.664	δ-3-Carene	---	1147	0.56
4.	5.175	α-Phellandrene	1217	1216	1.35
5.	8.370	3-Octanol	1340	1382	1.48
6.	10.184	L-Menthone	1401	1456	27.10
7.	10.362	*cis*-Sabinene hydrate	1407	1520	0.89
8.	10.961	L-Menthone	1426	1456	3.18
9.	12.918	L-Linalool	1487	1538	1.99
10.	13.869	β-Caryophyllene	1518	1617	0.49
11.	14.211	Isoneomenthone	1529	1622	4.13
12.	15.533	Menthol	1572	1612	20.25
13.	16.301	neo-Menthol acetate	1597	---	1.38
14.	17.139	*trans*-Anethole	1625	1809	1.24
15.	17.389	Piperitone oxide	1633	1700	3.41
16.	17.977	Piperitone oxide	1653	1700	6.48
17.	18.216	Butyloctadecanoate	1661	---	0.47
18.	24.584	Caryophyllene oxide	1883	1927	0.47
19.	27.778	2,5-Dimethyl-3-hexyne-2,5-diol	2001	---	0.56
20.	29.988	Thymol	2090	2115	1.49
21.	31.594	α-Aminoisobutanoic acid	2156	---	0.53
22.	39.075	18,18-Bi-1,4,7,10,13,16-	2482	---	0.49

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		Hexaoxacyclononadecane			
23.	40.227	18,18-Bi-1,4,7,10,13,16-Hexaoxacyclononadecane	2537	---	1.04
24.	40.333	(2S,2'S)-2,2-Bis[1,4,7,10,13-pentaoxacyclopentadecane]	2543	---	0.46
25.	40.550	(2S,2'S)-2,2-Bis[1,4,7,10,13-pentaoxacyclopentadecane]	2553	---	1.40
26.	40.675	2-Hydroxyhexadecyl-2,3-isopropylidene glycerol	2559	---	0.68
27.	40.792	Dodecyltriglycol	2565	---	1.30
28.	40.842	Dodecyltriglycol	2568	---	0.56
29.	40.922	Dodecyltriglycol	2572	---	1.21
30.	41.000	2-Hydroxyhexadecyl-2,3-isopropylidene glycerol	2575	---	0.69
31.	41.108	(2S,2'S)-2,2-Bis[1,4,7,10,13-pentaoxacyclopentadecane]	2581	---	0.73
32.	41.233	(2S,2'S)-2,2-Bis[1,4,7,10,13-pentaoxacyclopentadecane]	2587	---	0.97
33.	41.399	(2S,2'S)-2,2-Bis[1,4,7,10,13-pentaoxacyclopentadecane]	2595	---	1.90
34.	41.517	(2S,2'S)-2,2-Bis[1,4,7,10,13-pentaoxacyclopentadecane]	2601	---	0.45
35.	41.611	(2S,2'S)-2,2-Bis[1,4,7,10,13-pentaoxacyclopentadecane]	2605	---	0.86
36.	41.725	18,18-Bi-1,4,7,10,13,16-Hexaoxacyclononadecane	2610	---	0.66
37.	41.867	18,18-Bi-1,4,7,10,13,16-Hexaoxacyclononadecane	2616	---	0.47
38.	44.063	3-(1,3-Dihydroxyisopropyle)-1,5,8,11,14,17-hexaoxacyclononadecane	---	---	1.75
39.	44.965	18,18-Bi-1,4,7,10,13,16-Hexaoxacyclononadecane	---	---	0.97
40.	45.799	18,18-Bi-1,4,7,10,13,16-Hexaoxacyclononadecane	---	---	1.41

RT[a] : Retention time.
RI[b] : Retention indices according to their elution order on BP-20 polar column.
RI[c] : Actual retention indices of components on same phases of columns (BP-20, CW-20M, HP-20M and Supelcowax-10).
RA[d] : Percentage of components.
--- RI cannot calculate

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Table 4. Volatile oil composition of *M. arvensis* (MP) from Patiala (250m).

S. No.	RT[a]	Constituents	RI[b]	RI[c]	RA[d]
1.	4.021	dl-Limonene	---	1154	19.34
2.	4.140	p-Menth-2-en-1-ol	---	---	0.35
3.	4.245	Verbenene	---	1123	0.12
4.	4.573	α-Pinene	---	1039	0.29
5.	4.775	δ-3-Carene	1200	1147	0.08
6.	4.910	δ-3-Carene	1205	1147	0.12
7.	5.536	α-Terpinolene	1232	1287	0.09
8.	6.946	Octyl cyclobutanecarboxylate	1290	---	0.08
9.	8.564	3-Octanol	1347	1382	0.31
10.	10.204	Limonene oxide	1402	---	0.11
11.	10.578	*trans*-Sabinene hydrate	1414	1465	0.17
12.	11.848	β-Bourbonene	1454	1496	0.93
13.	13.163	L-Linalool	1495	1538	0.30
14.	13.439	Germacrene-D	1445	1613	0.11
15.	13.961	1-Methyl-5-methylene-8-(1-methylethyl)-1,6-cyclodecadiene	1521	---	0.12
16.	14.097	*trans*-Caryophyllene	1525	---	1.46
17.	14.683	*trans*-Dihydrocarvone	1544	1600	6.36
18.	15.171	*trans*-Dihydrocarvone	1560	1600	0.33
19.	15.437	Germacrene-D	1569	1613	0.27
20.	16.242	α-Humulene	1595	1563	0.10
21.	16.342	Epi-Bicyclosesquiphellandrene	1599	---	0.24
22.	16.584	Dihydrocarvyl acetate	1607	1657	0.94
23.	17.477	Germacrene-D	1636	1613	2.37
24.	17.629	*cis*-Piperitone oxide	1641	1700	0.19
25.	18.150	Piperitone	1659	1697	0.28
26.	18.564	Carvone	1672	1684	60.25
27.	18.799	1,2,3,4,4a,5,6,8a-Octahydro-7-methyl-4-methylene-1-(1-methylethyl)-naphthalene	1680	---	0.14
28.	18.865	β-Elemene	1682	1596	0.16
29.	18.989	Dihydrocarveol	1687	1713	1.02

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30.	19.404	2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-ol-acetate	1700	---	0.36
31.	20.833	Calamenene	1750	1826	0.13
32.	21.242	*trans*-Carvone oxide	1764	---	0.11
33.	21.358	1-Carveol	1768	1790	1.62
34.	22.166	*cis*-Carveol	1796	1869	0.29
35.	23.460	Piperitenone	1843	1851	0.28
36.	24.375	Piperitenone oxide	1876	1945	0.10
37.	26.742	Germacrene-D	1963	1613	0.11
38.	26.877	Cubenol	1968	1633	0.14
39.	29.765	Eugenol	2081	2103	0.08
40.	31.159	α-Cadinol	2138	2180	0.15

RT[a] : Retention time.
RI[b] : Retention indices according to their elution order on BP-20 polar column.
RI[c] : Actual retention indices of components on same phases of columns (BP-20, CW-20M, HP-20M and Supelcowax-10).
RA[d] : Percentage of components.
--- : RI cannot calculate

Figure 1. *GCMS Chromatogram of M. arvensis (M-1) collected from Fatehpur (415m). H.P.*

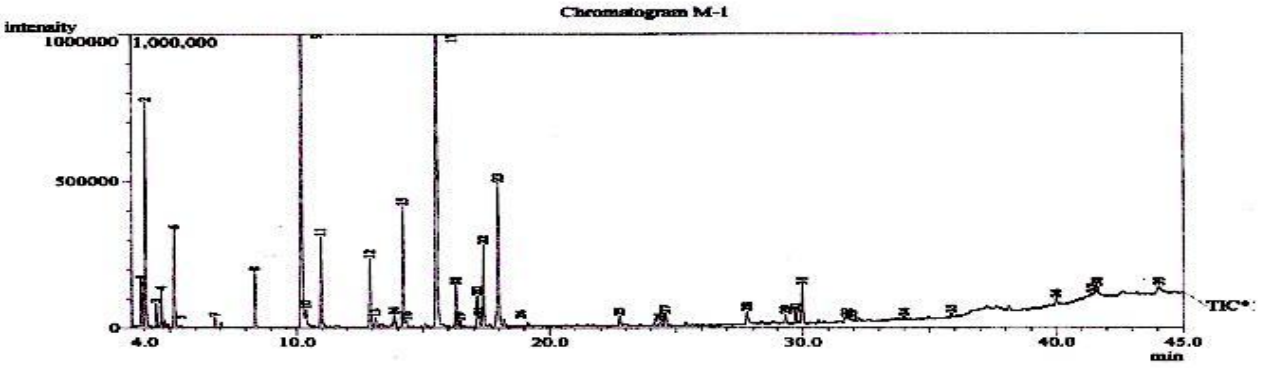

Figure 2. *GCMS Chromatogram of M. arvensis (M-2) collected from Dhameta (415m) H.P.*

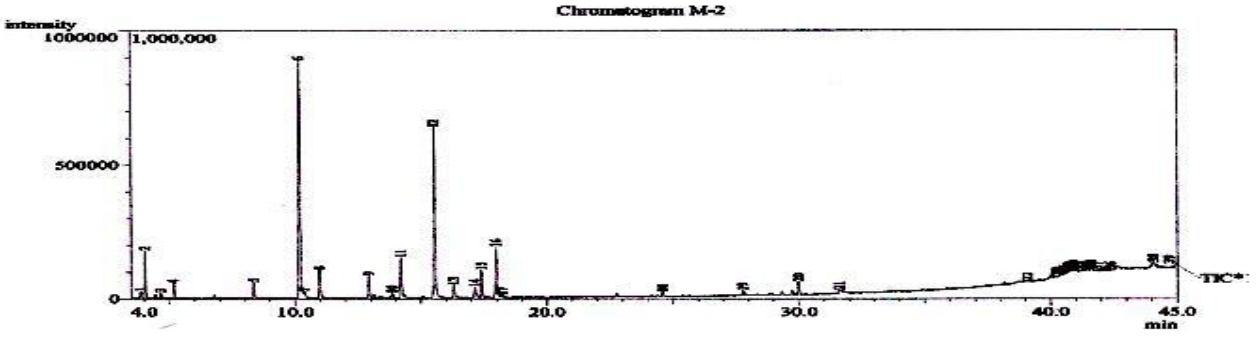

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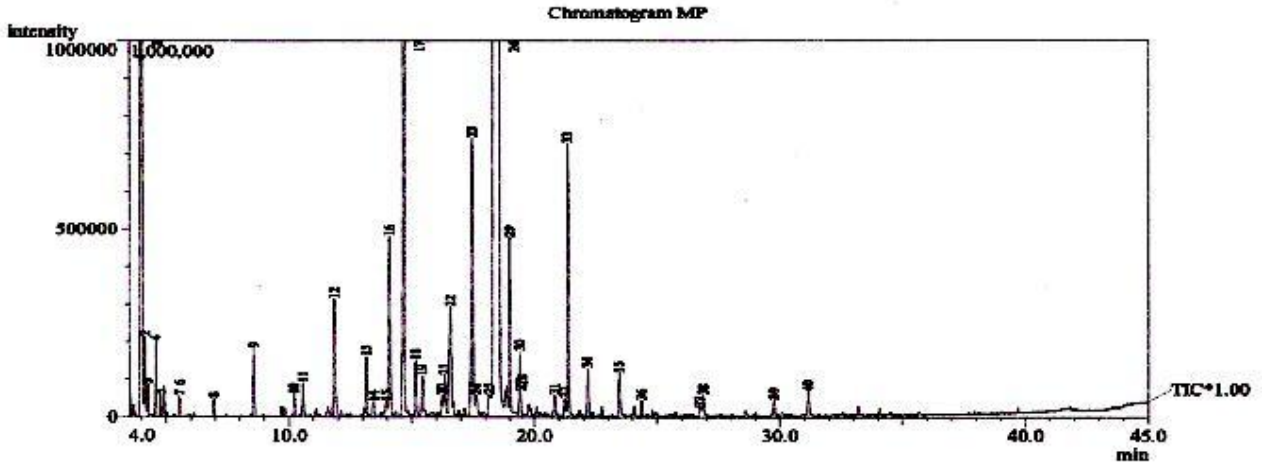
Figure 3. *GCMS Chromatogram of M. arvensis (MP) collected from Patiala (250m) Punjab.*



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