Results and Discussion

3.1 *Hibiscus asper oil*

The gas chromatography mass spectrometry analysis of *Hibiscus asper* oil was conducted and the identification of the constituents was accomplished by retention times and MS fragmentation pattern. A 90-95% match was observed when comparing the mass spectra with the database on MS library.

3.1.1 Constituents of *Hibiscus asper* oil

The GC-MS spectrum of the studied oil revealed the presence of 16 constituents (Table 3.1).The typical total ion chromatograms (TIC) is depicted in Fig (3.1) .The major constituents of the oil are:

i) 9, 12-Octadecadienoic acid (z,z) methyl ester (41.34 %)

ii) 9-Octadecenoic acid (z) methyl ester (25.90 %)

iii) Hexadecanoic acid, methyl ester (20.84%)

iv) Methyl stearate (06.67%)

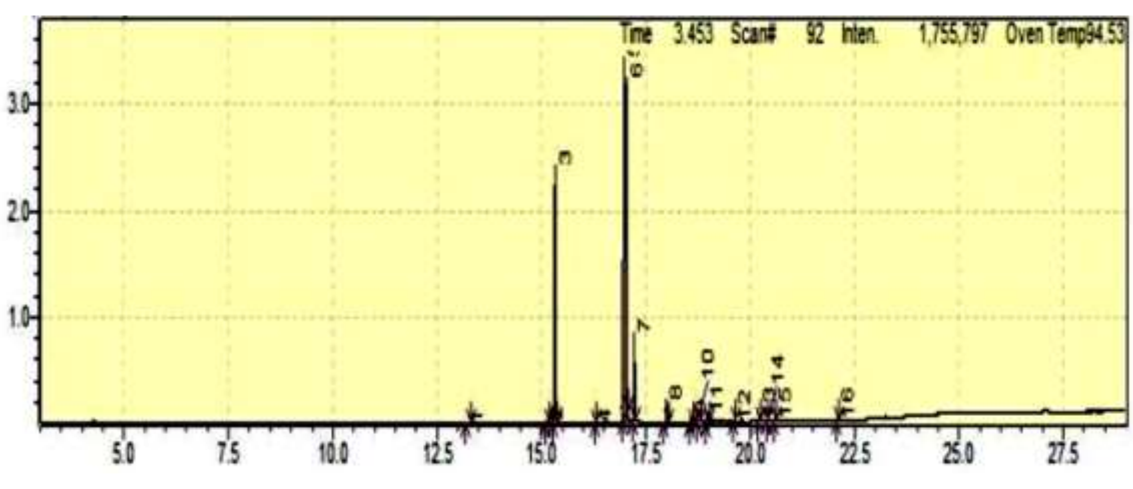

Fig.3.1: Chromatogram of *Hibiscus asper* oil

Table .3.1: Constituents of *Hibiscus asper* oil

ID#	Name	Ret.Time	Area	Area%
1.	Methyl tetradecanoate	13.227	261033	0.13
2.	9-Hexadecenoic acid, methyl ester, (Z)-	15.120	338367	0.17
3.	Hexadecanoic acid, methyl ester	15.322	42225774	20.84
4.	Heptadecanoic acid, methyl ester	16.289	137205	0.07
5.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	16.985	83755167	41.34
6.	9-Octadecenoic acid (Z)-, methyl ester	17.029	52480010	25.90
7.	Methyl stearate	17.222	13519511	6.67
8.	10-Octadecenoic acid, methyl ester	17.993	3552938	1.75
9.	13-Tetradece-11-yn-1-ol	18.580	1517499	0.75
10	Oleic Acid	18.748	463366	0.23
11	Eicosanoic acid, methyl ester	18.975	1496187	0.74
12	Octadecanoic acid, 9,10-dihydroxy-, methyl ester	19.629	255119	0.13
13	(Z)6,(Z)9-Pentadecadien-1-ol	20.212	1251810	0.62
14	15-Tetracosenoic acid, methyl ester	20.417	327933	0.16
15	Docosanoic acid, methyl ester	20.595	601289	0.30
16	Triacontanoic acid, methyl ester	22.097	414901	0.20
			202598109	100.00

The major constituents are discussed below:

(i) 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (41.34%)

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig.3.2. The peak at m/z294, which appeared at R.T. 16.985 in total ion chromatogram, corresponds to $M^{+}[C_{19}H_{34}O_{2}]^{+}$ .The peak at m/z263 corresponds to loss of a methoxyl function.

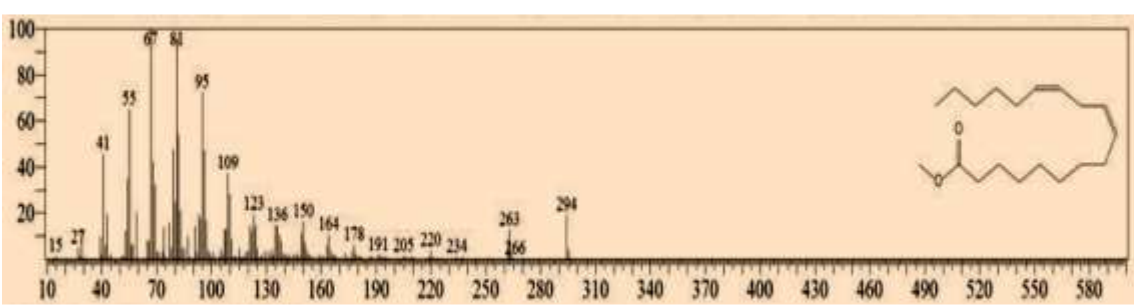

Fig.3.2: Mass spectrum of 9,12-octadecadienoic acid methyl ester

9,12-Octadecadienoic (linoleic acid) belongs to one of the two families of essential fatty acids. Such acids cannot be synthesized by human bodies and are available through diet. Linoleic acid is used in the biosynthesis of arachidonic acid . It exists in lipids of cell membrane. Rich sources are: nuts and fatty seeds. 9,12- Octadecadienoic is converted by some enzymes like lipoxygenases into mono-hydroxy products which are enzymatically oxidized to keto metabolites. Such metabolites are implicated in human physiology and pathology. Linoleate deficiency caused mild skin scaling, hair loss and poor wound healing in model animals [90-92].

(ii) 9-Octadecenoic acid (Z)-, methyl ester (25.90%)

The EI mass spectrum of 9-octadecenoic acid methyl ester is depicted in Fig.3.3. The signal which appeared at m/z 296 (R.T. 17.0290 corresponds to $M^+[C_{19}H_{36}O_2]^+$. The peak at m/z266 is due to loss of a methoxyl.

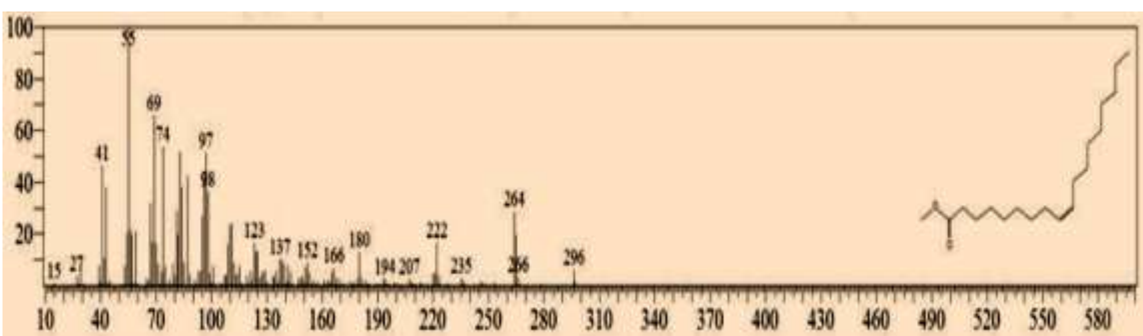

Fig.3.3: Mass spectrum of 9-octadecenoic acid methyl ester

As part of animal fats and vegetables, 9- octadecenoic acid(oleic acid) is included in the normal human diet. It is used as emollient. It is also a major component of soap. The acid is used in small amounts as excipient in pharmaceutical industries and as soldering flux in stained glass work. Oleic acid is a common monounsaturated fat in human diet. This acid may be responsible for the hypotensive potential of olive oil. The consumption of oleate in olive oil has been associated with decreased risk of breast cancer[93-95]

(iii) Hexadecanoic acid, methyl ester (20.84%)

The mass spectrum of hexadecanoic acid methyl ester is shown in Fig.3.4. The molecular ion $M^+[C_{17}H_{34}O_2]^+$ appeared at m/z 270. The peak at m/z239 is due to loss of a methoxyl group.

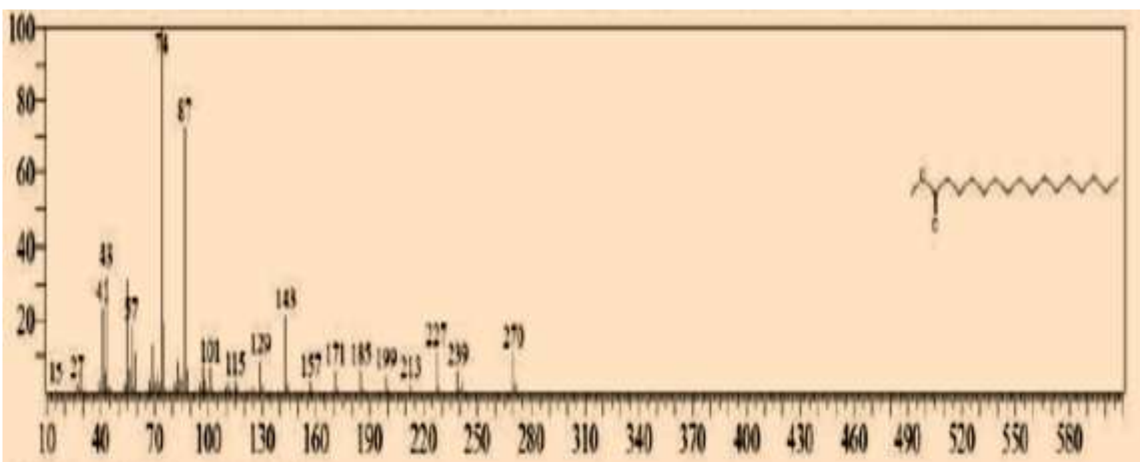

Fig.3.4: Mass spectrum of hexadecanoic acid methyl ester

Hexadecanoic acid (palmitic acid) is a saturated fatty acid . In plants and humans ,it is the most common fatty acid. During the synthesis of fatty acids, palmitic acid is produced first [96]. It is the precursor of long-chain fatty acids. This acid is a major lipid component of human breast milk[97]**.** Palmitic acid is used in production of soaps and cosmetics, it is also widely used in food industry .

(iv) Methyl stearate (6.67 %)

The EI mass spectrum of methyl stearate is shown in Fig.3.5. The peak at m/z 298 (R.T. 17.222 in total ion chromatogram) corresponds to $M^{+}[C_{19}H_{38}O_{2}]^{+}$ .The peak at m/z267 account for loss a methoxyl.

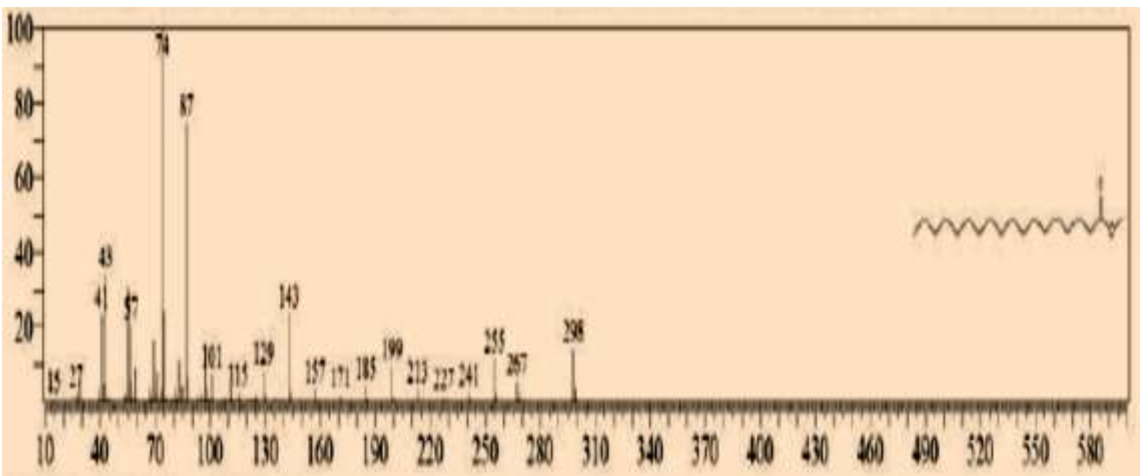

Fig.3.5: Mass spectrum of methyl stearate

Methyl stearate (methyl octadecanoate) is a white solid substance with oily waxy appearance and melting point (37 to 41^{o}c), and boiling (181 to 182^{o}c) and soluble in alcohol, but in water approximately 0.001 mg/l, it used as emollients, skin conditioning and in many others chemical products such as: washing & cleaning products, air care products and polishes, waxes, detergents paints and coating or adhesives, fragrances and air fresheners[98].

3.1.2 Antibacterial activity

In cup plate agar diffusion assay, the oil was evaluated for antimicrobial activity. The averages of the diameters of the growth inhibition zones are shown in Table (3.2). The results were interpreted as follow; <9mm: inactive; 9-12mm: partially active; 13-18mm: active; >18mm: very active). The sample showed partial activity against *Staphylococcus aureus,* and *Escherichia coli.* Tables (3.3) and (3.4) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

Table 3.2: Antibacterial activity of *Hibiscus asper* oil

standard	Bs.	Sa.	Pa.	Ec.	Ca.
Oil (100 mg/ml)	-	13	-	10	8

Table 3.3: Antibacterial activity of Standard chemotherapeutic agents

Drug	Conc. (mg/ml)	Bs.	Sa.	Ec.	Pa.
Ampicillin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-
Gentamycin	40	25	19	22	21
	20	22	18	18	15
	10	17	14	15	12

Table 3.4: Inhibition zones (mm) of standard drug

Standard	Conc. mg/ml	An.	Ca.
Clotrimazole	30	22	38
	15	17	31
	7.5	16	29

Bs.: *Bacillus subtilis* Sa.: *Staphylococcus aureus*

Ec.: *Escherichia coli* Pa.: *Pseudomonas aeruginosa*

An.: *Aspergillus niger* Ca.: *Candida albicans*

3.2 Merremia dissecta

3.2.1 Gas chromatography and mass spectrometry analysis

The gas chromatography mass spectrometry analysis of *Merremia dissecta* oil was conducted. The analysis revealed the presence of 20 components (Table3.5.) The typical total ion chromatograms (TIC) is shown in Fig(3.6).

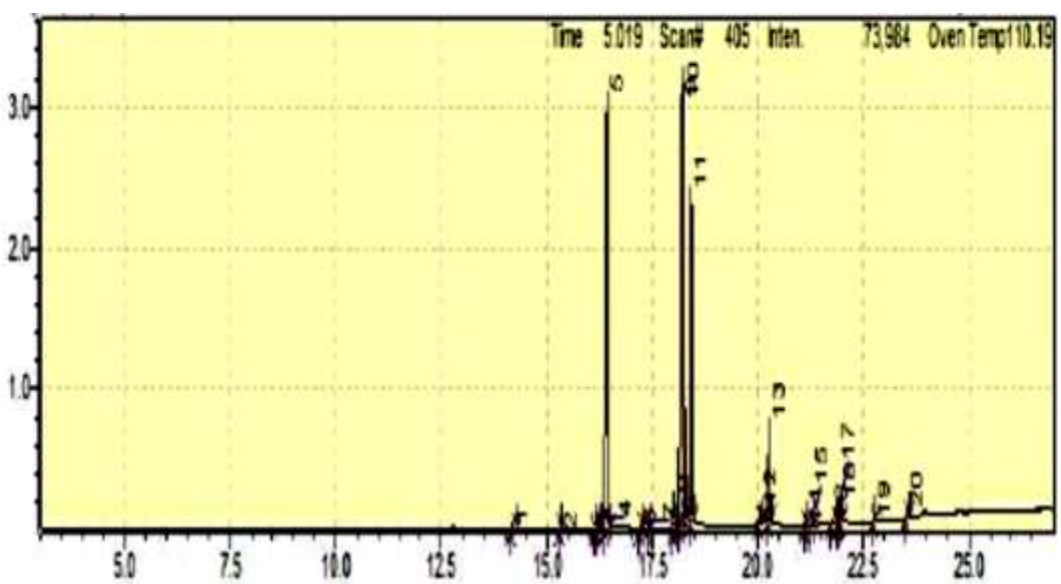

Fig 3.6. Chromatograms of *Merremia dissecta* oil

Table 3.9. Constituents of *Merremia dissecta* oil

ID#	Name	Ret.Time	Area	Area%
1.	Methyl tetradecanoate	14.187	1001016	0.26
2.	Pentadecanoic acid, methyl ester	15.317	148225	0.04
3.	7-Hexadecenoic acid, methyl ester, (Z)-	16.165	138376	0.04
4.	9-Hexadecenoic acid, methyl ester, (Z)-	16.194	1616242	0.42
5.	Hexadecanoic acid, methyl ester	16.420	88746045	23.04
6.	cis-10-Heptadecenoic acid, methyl ester	17.211	317146	0.08
7.	Heptadecanoic acid, methyl ester	17.424	1014215	0.26
8.	.gamma.-Linolenic acid, methyl ester	18.007	5222197	1.36
9.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.189	126215622	32.78
10	9-Octadecenoic acid (Z)-, methyl ester	18.240	84908512	22.04
11	Methyl stearate	18.428	50559962	13.12
12	cis-11-Eicosenoic acid, methyl ester	20.055	2682726	0.70
13	Eicosanoic acid, methyl ester	20.259	13537822	3.51
14	Heneicosanoic acid, methyl ester	21.126	282233	0.07
15	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	21.290	205759	0.05
16	cis-9-Hexadecenal	21.754	1039371	0.27
17	10,13-Eicosadienoic acid, methyl ester	21.912	1352185	0.35
18	Docosanoic acid, methyl ester	21.962	3397232	0.88
19	Tricosanoic acid, methyl ester	22.766	547438	0.14
20	Tetracosanoic acid, methyl ester	23.540	2289571	0.59
			385221895	100.00

The major constituents of the oil are:

(i) 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (32.78%)

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig.3.7. The peak at m/z294 (R.T. 18.189) coincides with $M^+[C_{19}H_{34}O_2]^+$, while the peak at m/z263 is due to loss of a methoxyl.

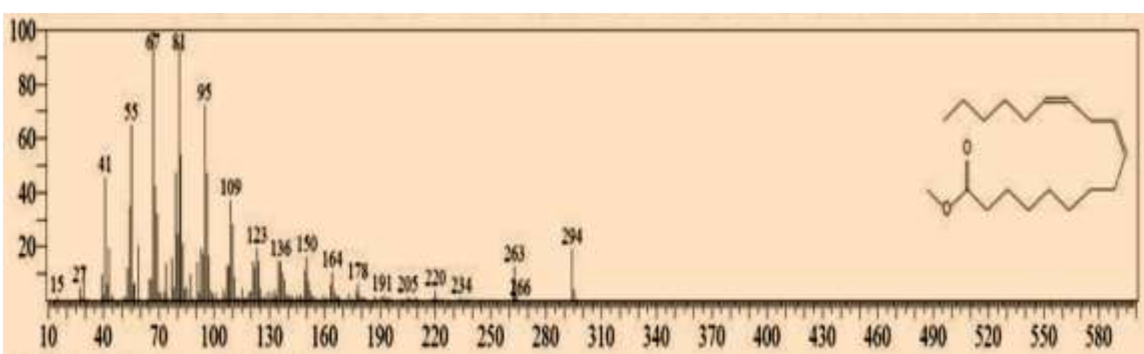

Fig.3.7. Mass spectrum of 9,12-octadecadienoic acid methyl ester

(ii) Hexadecanoic acid, methyl ester (23.04%).

Fig. 3.8 show The mass spectrum of hexadecanoic acid methyl ester. The signal at m/z 270, which appeared at R.T. 16.420 in total ion Chromatogram corresponds the molecular ion: $M^+[C_{17}H_{34}O_2]^+$. The peak at m/z239 is due to loss of a methoxyl.

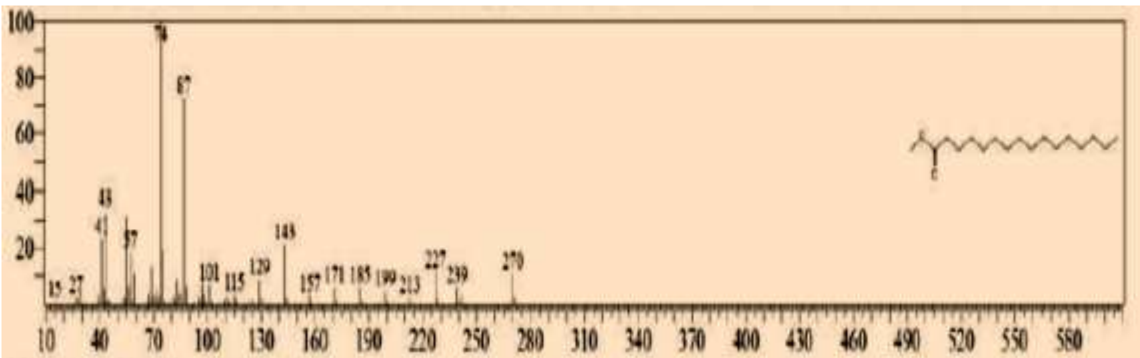

Fig.3.8: Mass spectrum of hexadecanoic acid methyl ester

(iii) 9-Octadecenoic acid (Z)-, methyl ester (22.04%)

The EI mass spectrum of 9-octadecenoic acid methyl ester is shown in Fig.3.9. The peak at m/z 296 (R.T. 18.240) is attributed to: $M^+[C_{19}H_{36}O_2]^+$ The signal at m/z266 account for loss of a methoxyl function.

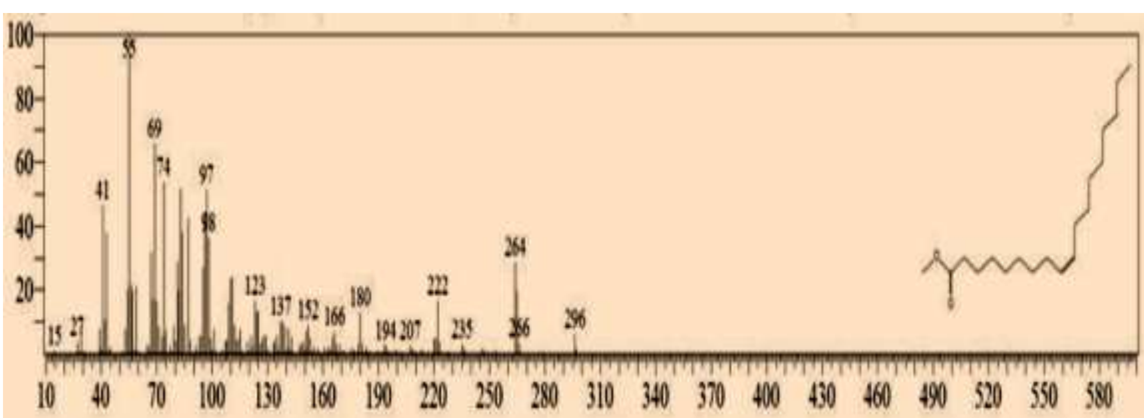

Fig.3.9: Mass spectrum of 9-octadecenoic acid methyl ester.

(iv) Methyl stearate (13.12 %)

The EI mass spectrum of methyl stearate is shown in Fig.3.10. The peak at m/z 298, which appeared at R.T. 18.428 in total ion chromatogram, corresponds to the molecular ion $M^{+}[C_{19}H_{38}O_{2}]^{+}$. The peak at m/z267 is attributed to loss of a methoxyl.

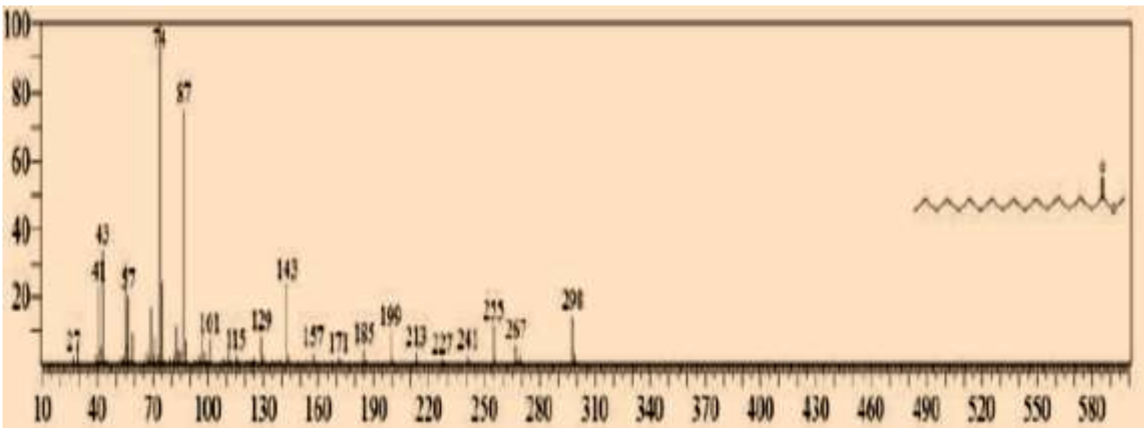

Fig.3.10: Mass spectrum of methyl stearate.

3.2.2 Antimicrobial activity

The oil was evaluated for antimicrobial activity against five microbial strains. The averages of the diameters of the growth inhibition zones are shown in Table(3.6). The sample showed an activity good against *Bacillus subtilis*, but it showed any response against others microbial strains

table 3.6. Antimicrobial activity of *Merremia dissecta*

Sample	Ec.	Pa.	Sa.	Bs.	Ca.
Merremia dissecta (100mg/ml)	-	-	-	15	-

3.3 *cucumis prophetarum*

3.3.1 Constituents of *cucumis prophetarum* oil

The oil from *cucumis prophetarum* has been analyzed by the hyphenated technique: GCMS. The analysis revealed the presence of 9 components (Table 3.7). The typical total ion chromatograms (TIC) is illustrated in Fig (3.11).

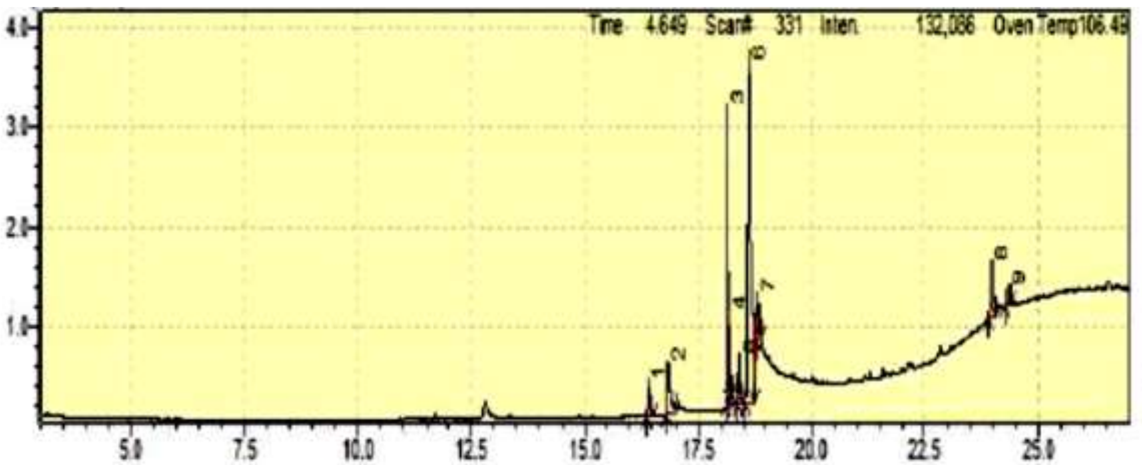

Fig 3.11. Chromatograms of *cucumis prophetarum* oil.

Table 3.7. Constituents of *cucumis prophetarum* oil.

ID#	Name	Ret.Time	Area	Area%
1.	Hexadecanoic acid, methyl ester	16.403	955776	2.75
2.	n-Hexadecanoic acid	16.846	2104757	6.06
3.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.144	5855223	16.86
4.	9-Octadecenoic acid (Z)-, methyl ester	18.183	1605102	4.62
5.	Methyl stearate	18.409	940149	2.71
6.	Linoleic acid ethyl ester	18.622	18842735	54.25
7.	Octadecanoic acid	18.822	1073244	3.09
8.	.gamma.-Sitosterol	23.976	2827700	8.14
9.	Squalene	24.329	528679	1.52
			34733365	100.00

The major constituents of the oil are:

(i) linoleic acid ethyl ester (54.25%)

Fig.3.12 illustrated the mass spectrum of linoleic acid ethyl ester. The signal at m/z280 (R.T. 18.622) coincides with $M^+[C_{18}H_{32}O_2]^+$ with the peak at m/z263 is due to loss of a methoxyl.

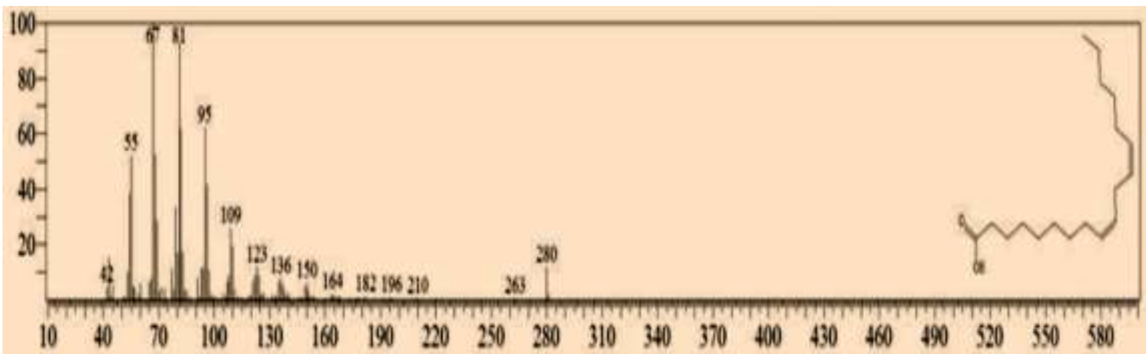

Fig.3.12. Mass spectrum linoleic acid ethyl ester

linoleic acid an essential fatty acid for human and animals. It has been known to be present in dairy products and other foods derived from ruminant animals, and it has been shown to have many biological activities (i.e. anticancer activity, immune-enhancing activity, weight-reducing effects and possible antiatherogenic properties) [99-100].

(ii) 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (16.86%)

The mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig.3.13. The peak at m/z294 (R.T. 18.144) is due to: $M^+[C19H34O2]^+$, while the peak at m/z263 account for loss of a methoxyl.

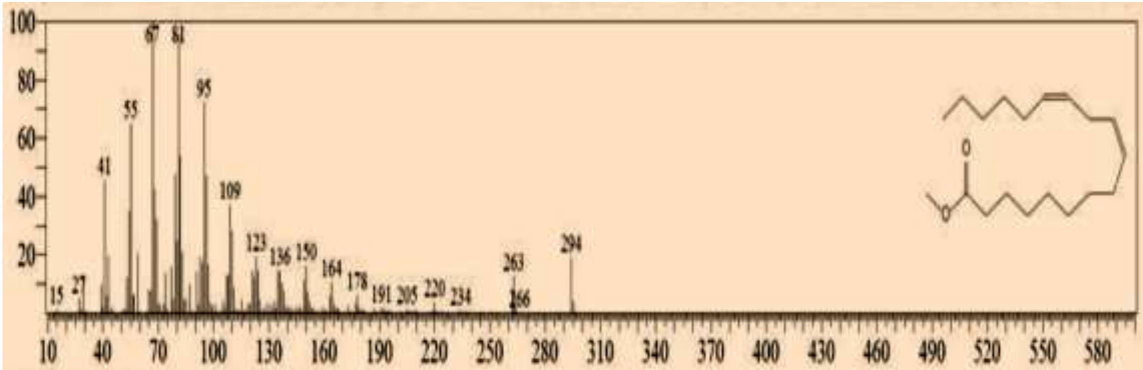

Fig.3.13. Mass spectrum of 9,12-octadecadienoic acid methyl ester

(iii) gamma.-Sitosterol (8.14 %)

Fig.3.14 shows the EI mass spectrum of gamma.-Sitosterol. The peak molecular ion $M^+[C_{29}H_{50}O]^+$ appeared at m/z414 (R.T. 23.976), while the peak at m/z396 is due to loss of a methoxyl.

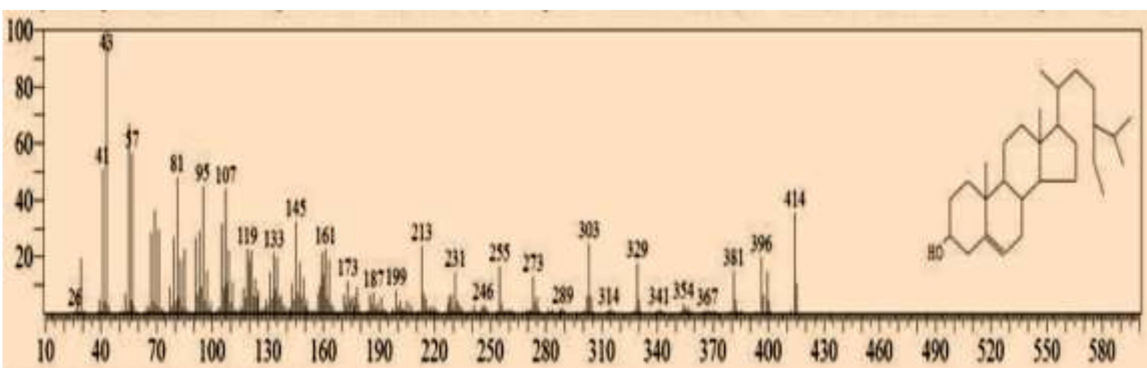

Fig.3.14. Mass spectrum of gamma.-Sitosterol

gamma-Sitosterol aplant steroid or phytosteroids, gamma-Sitosterol is an extremely weak basic (essentially neutral) compound. It can be used in Diabetes mellitus since it increases insulin secretion and inhibits glucogenesis[101-104].

(iv) n-Hexadecanoic acid (06.06 %).

The EI mass spectrum of n-Hexadecanoic acid is depicted in Fig.3.15. The peak at m/z 256 (R.T. 16.846) corresponds to $M^+[C_{16}H_{32}O_2]^+$. The signal at m/z 319 is due to loss of a methoxyl function.

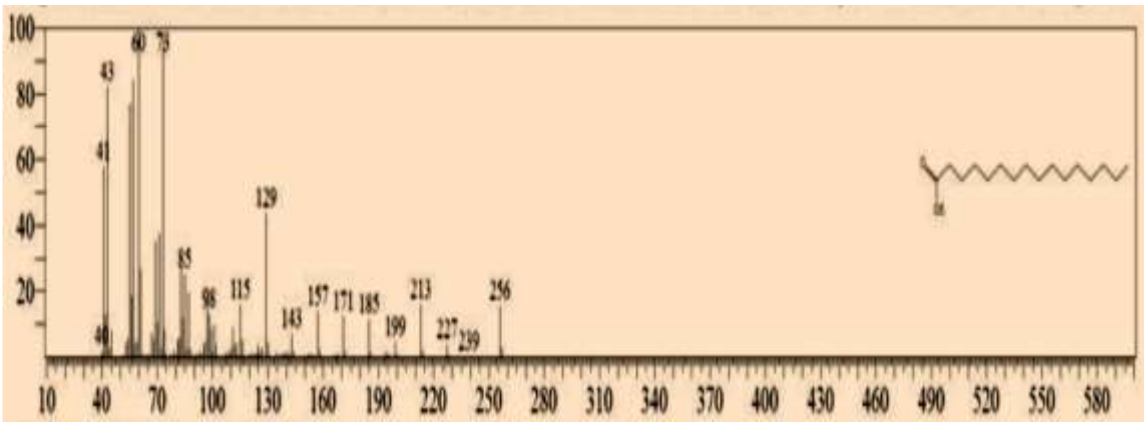

Fig.3.15: Mass spectrum of n-Hexadecanoic acid.

3.3.2-Antimicrobial activity

In cup plate agar diffusion bioassay, the oil was evaluated for antimicrobial activity. The averages of the diameters of the growth inhibition zones are shown in Table(3.8), but un fortunately the oil sample showed no response against the chosen five standard microbial.

Table 3.8. Antimicrbial activity of *cucumis prophetarum*.

Sample	Ec.	Pa.	Sa.	Bs.	Ca.
Merremia dissecta (100mg/ml)	-	-	-	-	-

3.4 *Citrullus lanatus*

3.4.1 Gas chromatography and mass spectrometry analysis

The GC-MS analysis of *Citrullus lanatus* oil was accomplished. The analysis revealed the presence of 20 components depicted in Table 3.9. The typical total ion chromatograms is also presented in Fig (3.16) below.

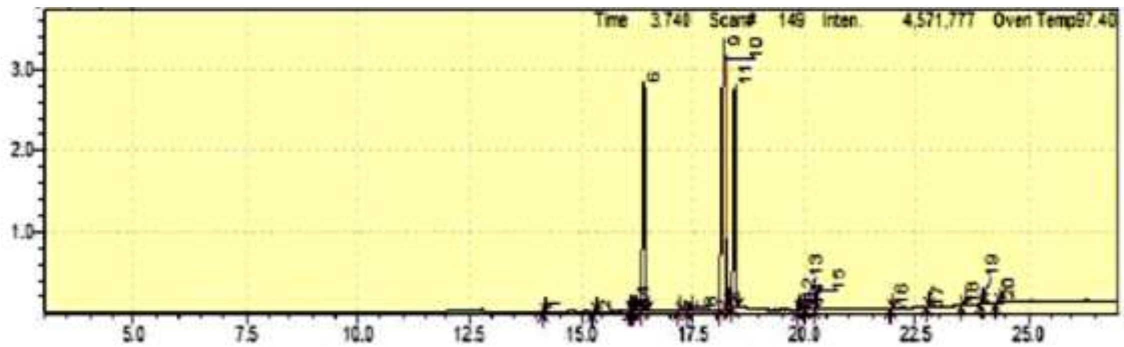

Fig 3.16. Chromatograms of *Citrullus lanatus* oil

Table 3.9. Constituents of *Citrullus lanatus* oil

ID#	Name	Ret.Time	Area	Area%
1.	Methyl tetradecanoate	14.173	483435	0.13
2.	Pentadecanoic acid, methyl ester	15.305	271220	0.07
3.	7,10-Hexadecadienoic acid, methyl ester	16.082	53537	0.01
4.	7-Hexadecenoic acid, methyl ester, (Z)-	16.145	58933	0.02
5.	9-Hexadecenoic acid, methyl ester, (Z)-	16.191	605600	0.16
6.	Hexadecanoic acid, methyl ester	16.410	68012932	18.10
7.	10-Octadecynoic acid, methyl ester	17.206	240113	0.06
8.	Heptadecanoic acid, methyl ester	17.420	811495	0.22
9.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.216	174051733	46.32
10	9-Octadecenoic acid (Z)-, methyl ester	18.241	49661613	13.22
11	Methyl stearate	18.436	69094607	18.39
12	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester	19.860	2991440	0.80
13	9-Octadecenoic acid, 12-hydroxy-, methyl ester, [R-(Z)]-	20.026	908092	0.24
14	cis-11-Eicosenoic acid, methyl ester	20.049	858753	0.23
15	Eicosanoic acid, methyl ester	20.254	4422904	1.18
16	Docosanoic acid, methyl ester	21.958	855722	0.23
17	Tricosanoic acid, methyl ester	22.763	247697	0.07
18	Tetracosanoic acid, methyl ester	23.535	763641	0.20
19	.gamma.-Sitosterol	23.977	588338	0.16
20	Squalene	24.328	707125	0.19
			375688930	100.00

The major constituents of the oil are:

(i) 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (46.32%)

Fig.3.17 presents The mass spectrum of 9,12-octadecadienoic acid methyl ester. The peak at m/z294 (R.T. 18.216) is due to the molecular ion: $M^+[C_{19}H_{34}O_2]^+$, while the peak at m/z263 is due to loss of a methoxyl.

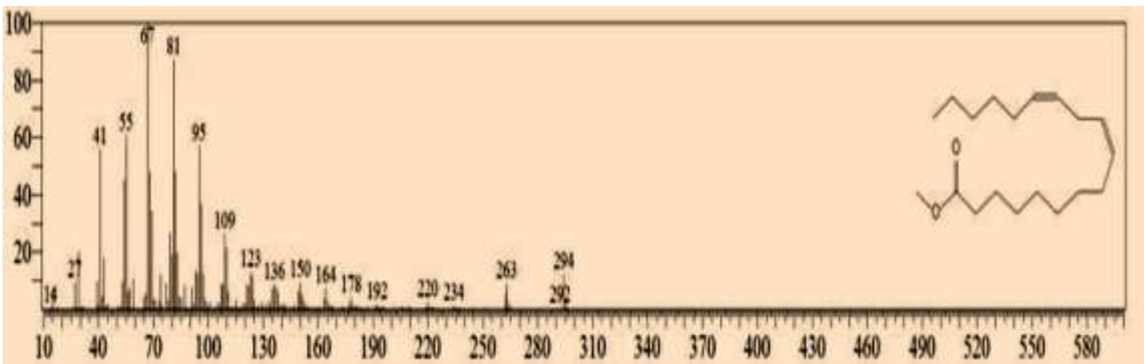

Fig.3.17. Mass spectrum of 9,12-octadecadienoic acid methyl ester

(ii) Methyl stearate (18.39 %)

The mass spectrum of methyl stearate is shown in Fig.3.18. The peak at m/z 298, which appeared at R.T. 18.436, corresponds: $M^+[C_{19}H_{38}O_2]^+$. The signal at m/z267 account for loss of a methoxyl group.

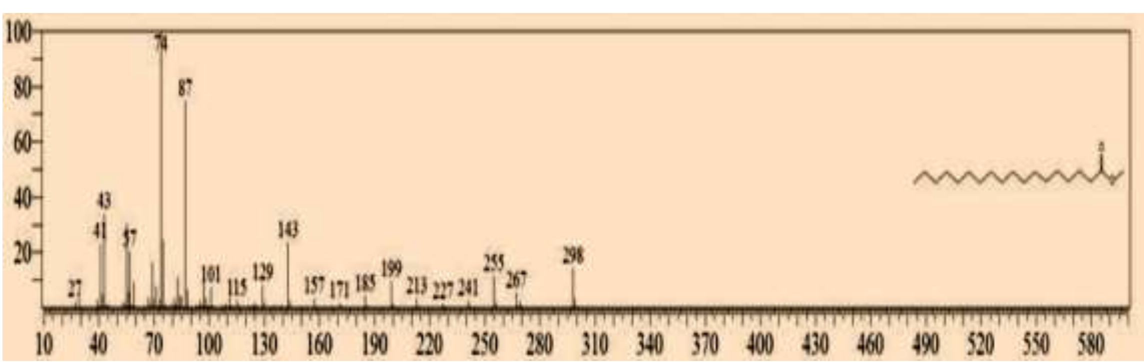

Fig.3.18: Mass spectrum of methyl stearate.

(iii) Hexadecanoic acid, methyl ester (18.10%)

Fig.3.19 illustrated the mass spectrum of hexadecanoic acid methyl ester. The molecular ion: $M^+[C_{17}H_{34}O_2]^+$ appeared at m/z 270 (R.T. 16.410). The peak at m/z239 is due to loss of a methoxyl.

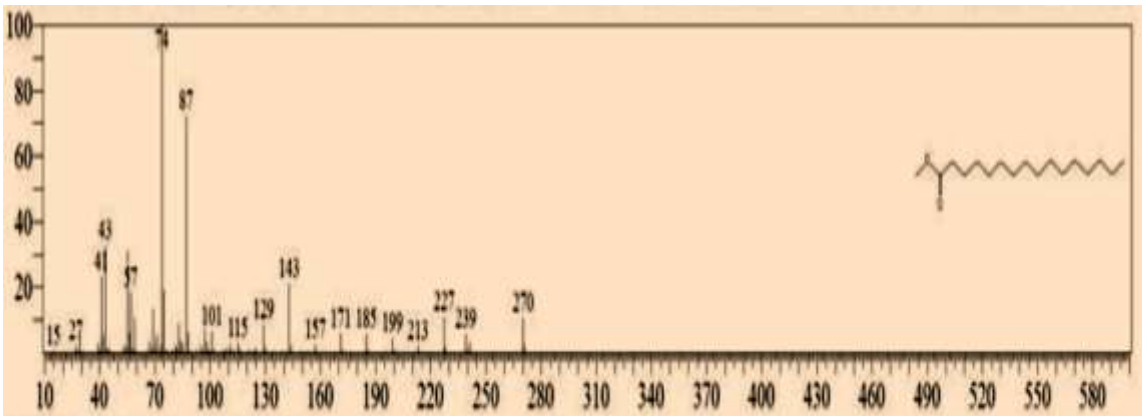

Fig.3.19: Mass spectrum of hexadecanoic acid methyl ester

(iv) 9-Octadecenoic acid (Z)-, methyl ester (13.22%)

The EI mass spectrum of 9-octadecenoic acid methyl ester is depicted in Fig.3.20. The signal at m/z 296 (R.T. 18.241) corresponds: $M^+[C_{19}H_{36}O_2]^+$ while the peak at m/z266 is attributed to loss of a methoxyl.

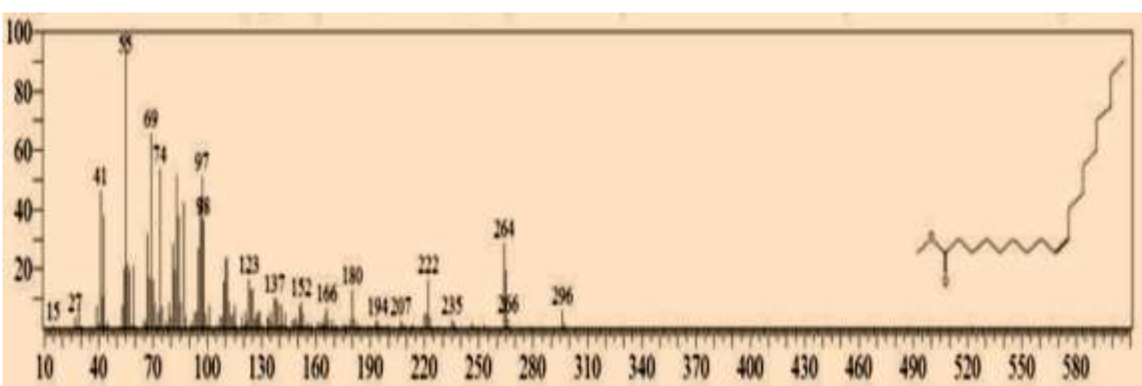

Fig.3.20: Mass spectrum of 9-octadecenoic acid methyl ester

3.4.2 Antimicrobial activity

The studied oil was screened for antimicrobial activity. The averages of the diameters of the growth inhibition zones are shown in Table(3.10).

Table 3.10. inhibition zones (mm) of *Citrullus lanatus* oil

Sample	Ec.	Pa.	Sa.	Bs.	Ca.
Citrullus lanatus (100mg/mL)	10	-	-	15	-

The oil showed good antibacterial activity against *Bacillus subtilis*, and partial activity against *Escherichia coli*.

3.5 *Cleome gynandra*

3.5.1 Gas chromatography and mass spectrometery analysis

GC-MS analysis of *Cleome gynandra* oil was revealed the presence of 17 components (Table 3.11.) The typical total ion chromatograms is also presented in Fig (3.21).

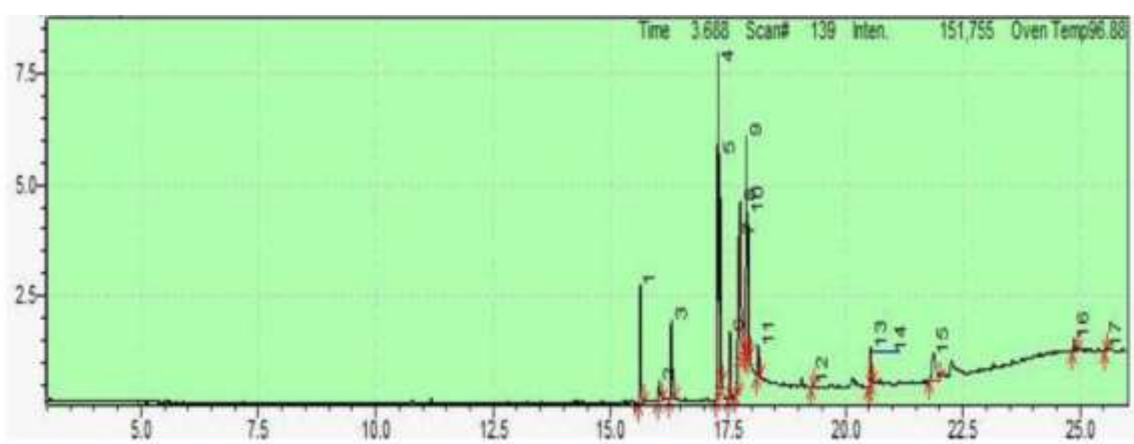

Fig 3.21. Chromatograms of Cleome gynandra oil

Table 3.11: Constituents of *Cleome gynandra* oil

Peak#	Name	R.Time	Area	Area%
1	Hexadecanoic acid, methyl ester	15.633	4297547	6.04
2	n-Hexadecanoic acid	16.032	1148327	1.61
3	Hexadecanoic acid, ethyl ester	16.295	2842955	4.00
4	9,12-Octadecadienoic acid (Z,Z)-, methyl e	17.287	14071589	19.78
5	9-Octadecenoic acid (Z)-, methyl ester	17.331	8133566	11.43
6	Methyl stearate	17.547	2346114	3.30
7	Linoleic acid ethyl ester	17.732	8651765	12.16
8	Oleic Acid	17.759	8073874	11.35
9	9,12-Octadecadienoic acid, ethyl ester	17.893	8447220	11.88
10	Ethyl Oleate	17.933	5122181	7.20
11	Octadecanoic acid, ethyl ester	18.146	1219951	1.72
12	Eicosanoic acid, methyl ester	19.304	157402	0.22
13	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	20.540	1418523	1.99
14	9-Octadecenoic acid, 1,2,3-propanetriyl est	20.555	728692	1.02
15	.gamma.-Sitosterol	21.877	3638376	5.12
16	.gamma.-Tocopherol	24.870	681003	0.96
17	Vitamin E	25.541	149940	0.21
			71129025	100.00

The major constituents of the oil are:

(i) 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (19.78%)

The mass spectrum of 9,12-octadecadienoic acid methyl ester is illustrated in Fig.3.22 . The signal at m/z294 (R.T. 17.287) coincides with the molecular ion: $M^+[C_{19}H_{34}O_2]^+$, while the peak at m/z263 is account for loss of a methoxyl.

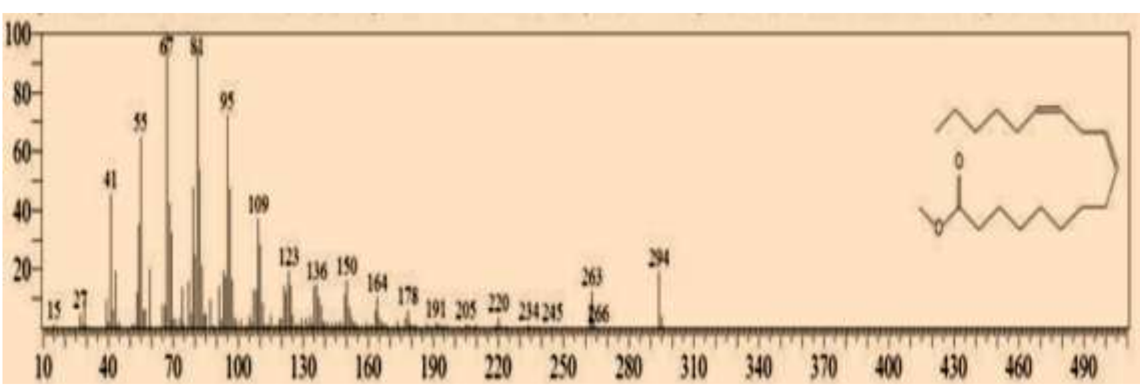

Fig.3.22. Mass spectrum of 9,12-octadecadienoic acid methyl ester

(ii) linoleic acid ethyl ester (12.16%)

Fig.3.23. illustrated the mass spectrum of linoleic acid ethyl ester. The molecular ion: $M^+[C_{18}H_{32}O_2]^+$ appeared at m/z280 (R.T. 17.732). The peak at m/z 263 is due to loss of a methoxyl.

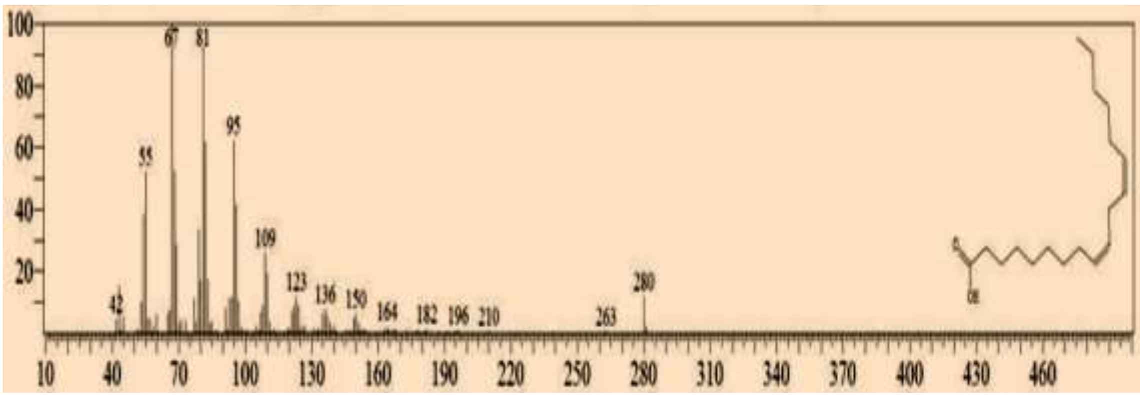

Fig.3.23. Mass spectrum linoleic acid ethyl ester

(iii) 9,12-Octadecadienoic acid, ethyl ester (11.88%)

The mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig.3.24 . The peak at m/z308 (R.T. 17.893) coincides with: $M^+[C_{20}H_{36}O_2]^+$, while the signal at m/z 279 is due to loss of a ethoxyl group.

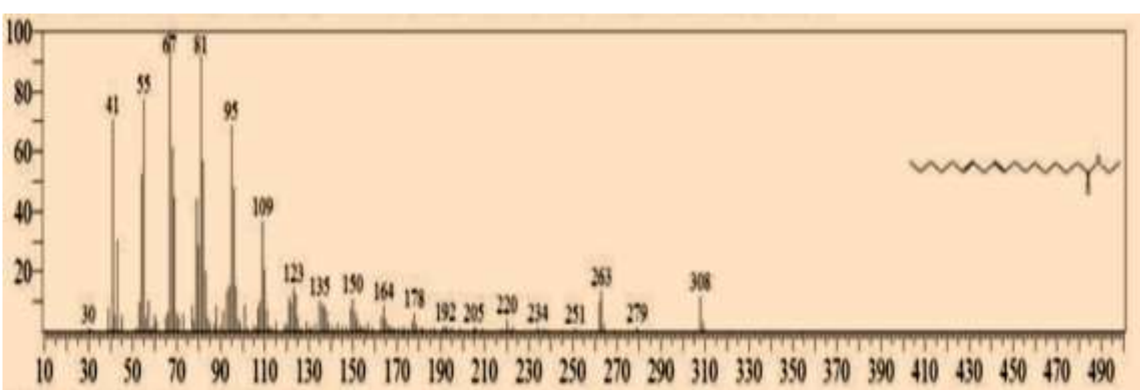

Fig.3.24. Mass spectrum of 9,12-octadecadienoic acid ethyl ester

Ethyl linoleate is a long-chain fatty acid ethyl ester resulting from the formal condensation of the carboxy group of linoleic acid with the hydroxy group of ethanol. It has a role as a plant metabolite and an anti-inflammatory agent. It derives from a linoleic acid[105].

(iv) 9-Octadecenoic acid (Z)-, methyl ester (11.43%)

Fig.3.25 illustrated the mass spectrum of 9-octadecenoic acid methyl ester. The molecular ion $M^+[C_{19}H_{36}O_2]^+$ appeared as a peak at m/z296 (R.T. 17.331). The signal at m/z266 is attributed to loss of a methoxyl group.

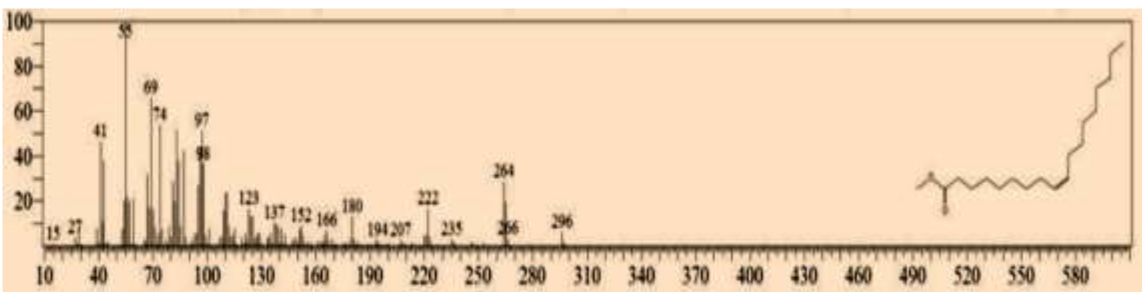

Fig.3.25: Mass spectrum of 9-octadecenoic acid methyl ester.

3.4.2 Antimicrobial activity

In cup plate agar diffusion bioassay, the oil was evaluated for antimicrobial activity. The averages of the diameters of the growth inhibition zones are shown in Table(3.12). The oil sample failed to show activity against the five standard organisms.

Table 3.12: Inhibition zones of *Cleome gynandra*

Sample	Ec.	Pa.	Sa.	Bs.	Ca.
Cleome gynandra (100mg/ml)	-	-	-	-	-