CHAPTER THREE

RESULTS AND DISCUSSIN

3- Results and Discussion

3.1-The GC-MS analysis of *Pithecellobium dulce* fixed oil

The oil from *Pithecellobium dulce* seeds was analyzed by GC-MS and the characterization of the constituents was initially accomplished by comparison with the MS library (NIST) and also confirmed by interpretation of the recorded fragmentation pattern.

The GC-MS analysis revealed the presence of 21 components (Table:3.1).The typical total ion chromatograms(TIC) of hexane extract are shown in Fig.3.1.

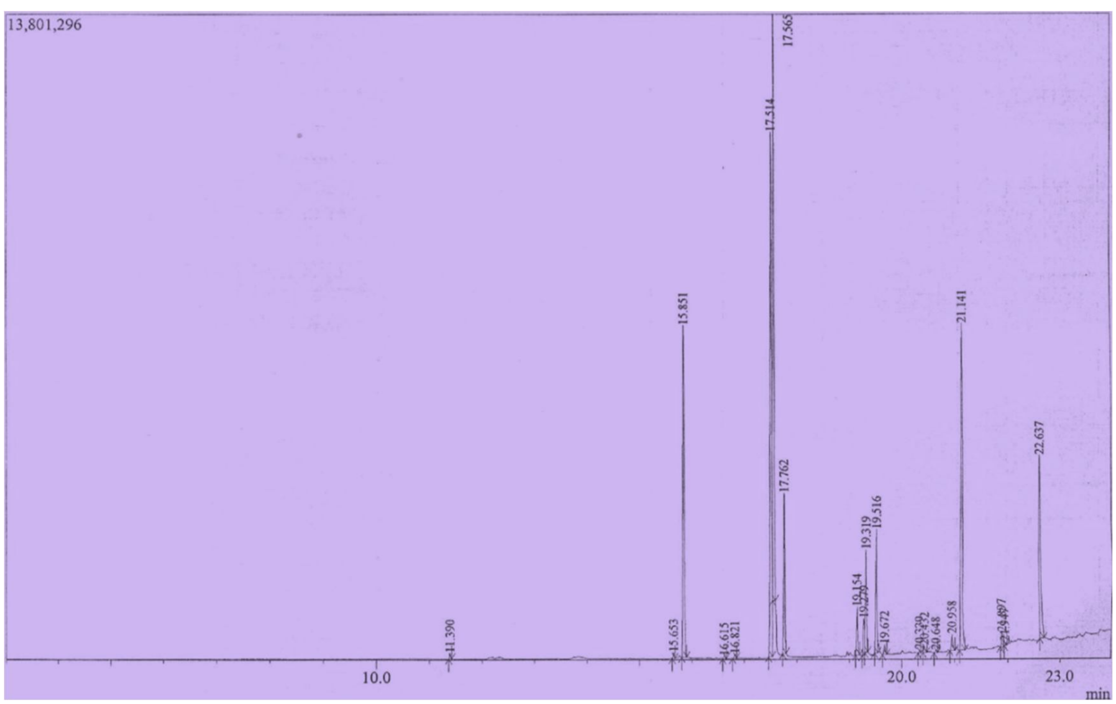

Fig.3.1: Total ion chromatograms

Table3.1 The typical total ionchromatograms(TIC

Peak#	R.Time	Area	Area%	Name
1	11.390	173449	0.17	Butylated Hydroxytoluene 9-
2	15.653	184499	0.18	Hexadecenoic acid, methyl ester, (Z)
3	15.851	12947477	12.88	Hexadecanoic acid, methyl ester
4	16.615	76299	0.08	cis-lO-Heptadecenoic acid, methyl ester
5	16.821	136513	0.14	Heptadecanoic acid, methyl ester
6	17.514	23724870	23.59	9,12-Octadecadienoic acid (Z,Z)-, methyl
7	17.565	22780049	22.65	9-Octadecenoic acid, methyl ester, (E)-
8	17.762	5816472	5.78	Methyl stearate
9	19.154	1676226	1.67	Tridecanedial
10	19.279	1355682	1.35	Oxiraneoctanoic acid, 3-octyl-,methyl
11	19.319	3585956	3.57	11-Eicosenoic acid, methyl ester
12	19.516	4354065	4.33	Methyl 18-methylnonadecanoate
13	19.672	390840	0.39	Methyl 15-hydroxy-9,12-octadecadienoate
14	20.339	91979	0.09	Heneicosanoic acid, methyl ester
15	20.432	248071	0.25	Phenol, 2,2•-methylenebis[6-(1,1-
16	20.648	96254	0.10	Octadecanoic acid, 2,3-dihydroxypropyl
17	20.958	636972	0.63	cis-lO-Nonadecenoic acid, methyl ester
18	21.141	14071742	13.99	Methyl 20-methyl-heneicosanoate
19	21.897	594787	0.59	Tricosanoic acid, methyl ester
20	21.947	124058	0.12	Oxiraneoctanoic acid, 3-octyl-, methyl est(
21	22.637	7490660	7.45	Tetracosanoic acid, methyl ester
		100556920	100.00	

Fatty acids constituted the major bulk of the oil and two antioxidants :butylated hydroxytoluene and 2,2`-Methylene-bis-[6-(1,1-dimethylethyl)-4-methyl]phenol were detected as minor constituents;0.17% and 0.25% respectively.

Some important constituents are discussed below:

Hexadecanoic acid methyl ester

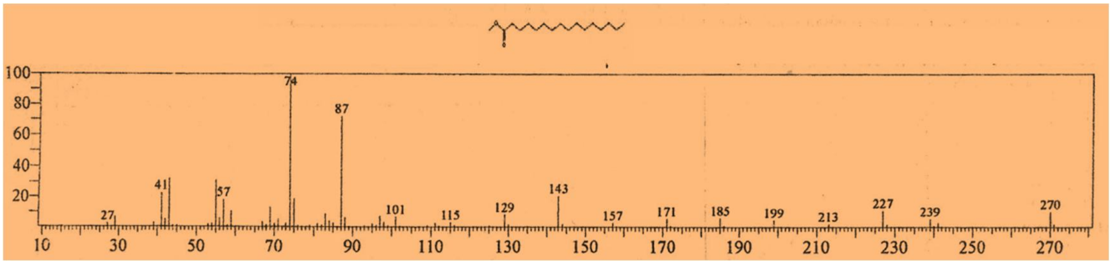

Fig. 3.2: Mass spectrum of hexadecanoic methyl ester

The EI mass spectrum of hexadecanoic acid methyl ester is shown in Fig. 3.2.The peak at m/z 270, which appeared at R.T. 13.308 in total ion chromatogram, corresponds to $M^{+}[C_{17}H_{34}O_{2}]^{+}$ .The peak at m/z239 corresponds to loss of a methoxyl function.

9,12-Octadecadienoic acid methyl ester(23.59%)

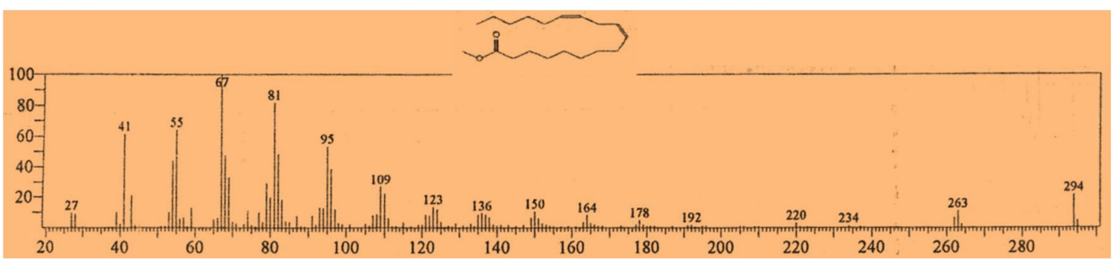

Fig. 3.3: Mass spectrum of 9,12-octadecanoic acid methyl ester

The EI mass spectrum of 9,12-octadecanoic acid methyl ester

is shown in Fig. 3.3.The peak at m/z 294, which appeared at R.T. 17.514 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.

9-Octadecenoic acid methyl ester(22.65%)

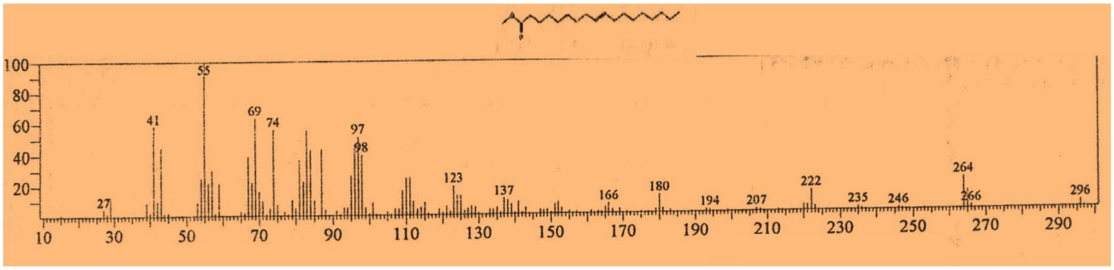

Fig. 3.4: Mass spectrum of 9-octadecanoic acid methyl ester

The EI mass spectrum of 9-octadecenoic acid methyl ester is shown in Fig. 3.4.The peak at m/z 296, which appeared at R.T. 17.565 in total ion chromatogram, corresponds to $M^{+}[C_{19}H_{36}O_{2}]^{+}$ .The peak at m/z265 corresponds to loss of a methoxyl function.

Methyl stearate(5.78%)

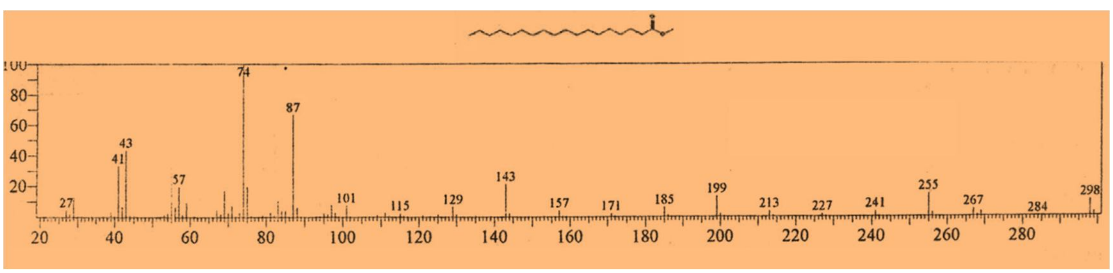

Fig. 3.5: Mass spectrum of methyl stearate

The EI mass spectrum of methyl stearate is shown in Fig. 3.5.The peak at m/z 298, which appeared at R.T. 17.762 in total ion chromatogram, corresponds to $M^{+}[C_{19}H_{38}O_{2}]^{+}$ .The peak at m/z267 corresponds to loss of a methoxyl function

Cis-11-Eicosenoic acid methyl ester(3.57%)

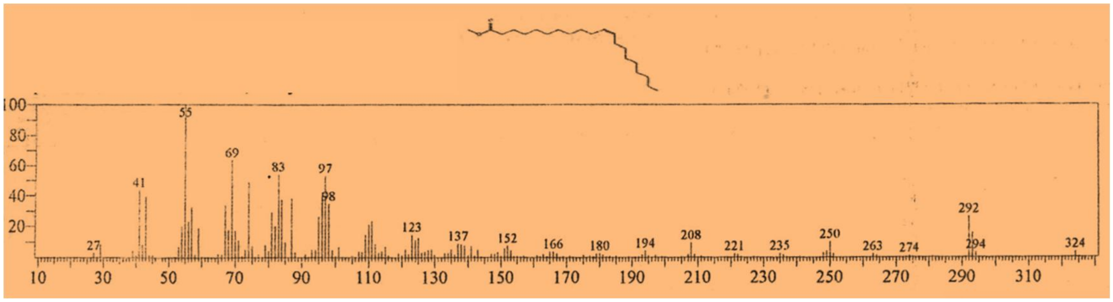

Fig. 3.6: Mass spectrum of Cis-11-Eicosenoic acid methyl ester

The EI mass spectrum of Cis-11-eicosenoic acid methyl ester is shown in Fig. 3.6.The peak at m/z324, which appeared at R.T. 19.319 in total ion chromatogram, corresponds to $M^+[C_{21}H_{40}O_2]^+$ .The peak at m/z293 corresponds to loss of a methoxyl function.

Butylated hydroxytoluene(0.17%)

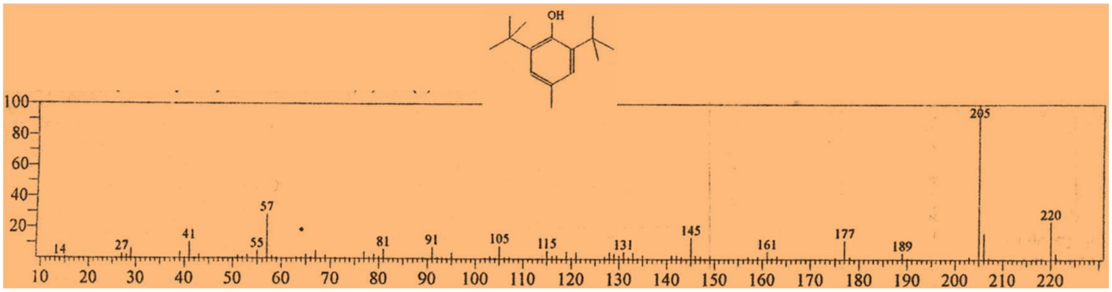

Fig. 3.7: Mass spectrum of butylated hydroxytoluene

The EI mass spectrum of butylated hydroxytoluene is shown in Fig. 3.7.The peak at m/z 220, which appeared at R.T. 11.390 in total ion chromatogram, corresponds to $M^+[C_{15}H_{24}O]^+$. The peak at 205 is due loss of a methyl function.

2,2`-Methylene-bis-[6-(1,1-dimethylethyl)-4-methyl]phenol(0.25%)

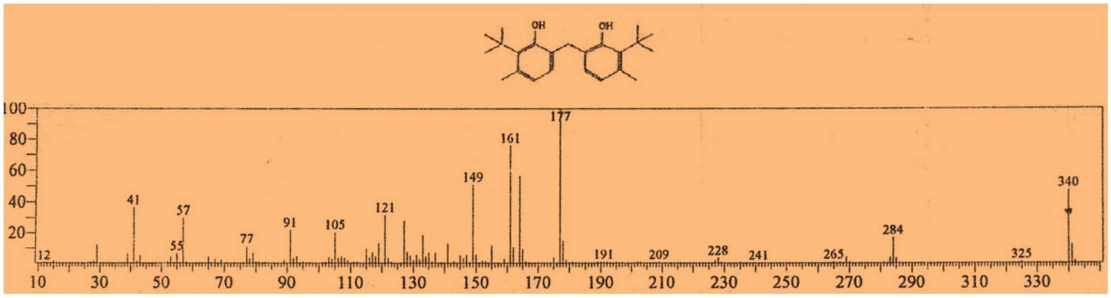

Fig. 3.8: Mass spectrum of 2,2`-Methylene-bis-[6-(1,1-dimethylethyl)-4-methyl]phenol

The EI mass spectrum of 2,2`-methylene-bis-[6-(1,1-dimethylethyl)-4-methyl]phenol is shown in Fig. 3.8.The peak at m/z340, which appeared at R.T. in total ion chromatogram, corresponds to $M^{+}[C_{15}H_{24}O]^{+}$ .The peak at m/z325 corresponds to loss of CH_3 .

3.2-Antimicrobial activity

The oil was evaluated for antimicrobial potency against standard organisms. The average of the diameters of the growth inhibition zones are shown in Table (3.3) .The results were interpreted in terms of the commonly used terms (<9mm: inative;9-12mm:partially active;13-18mm: active;>18mm:very active) .Tables (3.4) and (3.5) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

Table (3.3) : Antibacterial activity of *Pithecellobium dulce* oil :M.D.I.Z (mm)

Drug	Conc.(mg/ml)	Ec	Ps	Sa	Bs	Ca	An
oil	100	-	-	8	11	11	10

Table (3.4) : Antibacterial activity of standard chemotherapeutic agents :M.D.I.Z (mm)

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

Table (3.5) : Antifungal activity of standard chemotherapeutic agents against standard fungi

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

- Sa.: *Staphylococcus aureus*
- Ec.: *Escherichia coli*
- Pa.: *Pseudomonas aeruginosa*
- An.: *Aspergillus niger*
- Ca.: *Candida albicans*
- *Bs.: Bacillus subtilis*
- M.D.I.Z: Mean diameter or growth inhibition zone (mm)..

The oil showed partial activity against *Bacillus subtilis* and the fungi *Candida albicans* and *Aspergillus niger*.However, it was inactive against other test organisms.

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