3-Results and Discussion

The ethanol extract from *Aristolochia bracteolata* was fractionated by paper chromatography. After the usual workup compound I(which appeared blue under UV light) and compound II (which appeared yellow under UV light)were isolated from *Aristolochia bracteolate* leaves.

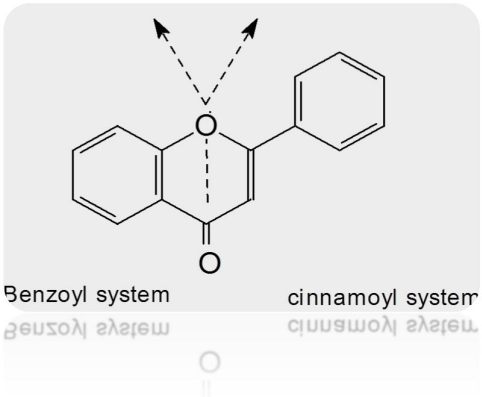
3.1-Phytochemical screening

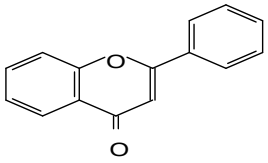
Phytochemical screening of leaves revealed the presence of many secondary metabolites : alkaloids, flavonoids, steroids, terpenoids, tannins and saponins.

3.2- Spectral data of compound I

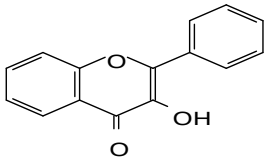
In their UV spectra flavones,flavonols,chalcones and aurones give both band I(due to cinnamoyl chromophore) and band II(due to benzoyl chromophore).

Other classes: isoflavones, flavanones, dihydrochalcones and dihydroflavonols show only one peak originating from the benzoyl system. Band I , usually 300 – 400nm and band II ,usually 240 – 280 nm126 .

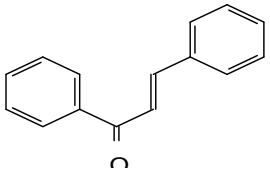




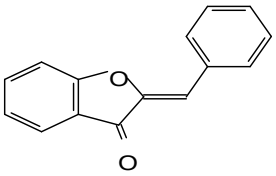
Flavone



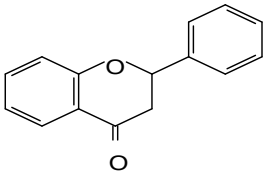
Flavonol



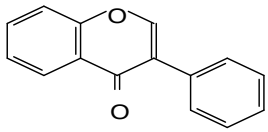
Chalcone



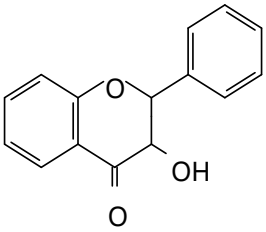
Aurone



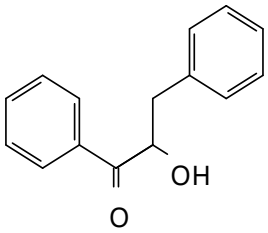
Flavanone



Isoflavone



Dihydroflavonol



Dihydrochalcone

The UV absorption of some important classes of flavonoids is depicted in table 4.

Table 4: The UV absorption of some flavonoids

Flavonoid class	Band I	Band II
Favone	330-350	250-270
Flavonol	350-390	250-280
Chalcone	365-390	240-260
Aurone	390-430	240-270

In UV , compound I absorbs(Fig.1) at λ_{max} (MeOH) 285 nm.Such absorption is given by: i) flavanones ii) isoflavones iii)dihydroflavonols and iv)dihydrochalcones. However, no shoulder characteristic of isoflavones was detected in the range : 300-340nm(Fig.1). Furthermore , the double multiplets which characterize flavanones and which appear at δ2,80 and δ5.20ppm was not detected in the ^{1}HNMR spectrum(Fig.). The sodium methoxide spectrum(Fig.2) revealed a bathochromic shift dignostic of dihydroflavonols.

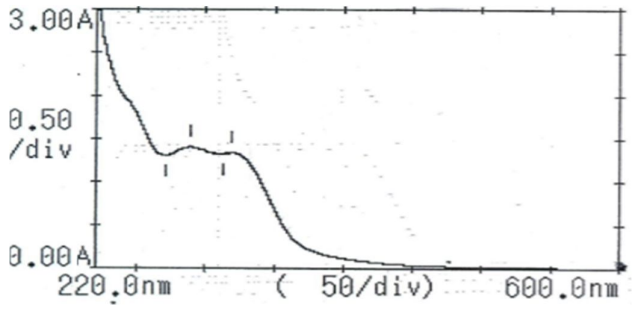


Fig.1 : UV spectrum of compound I

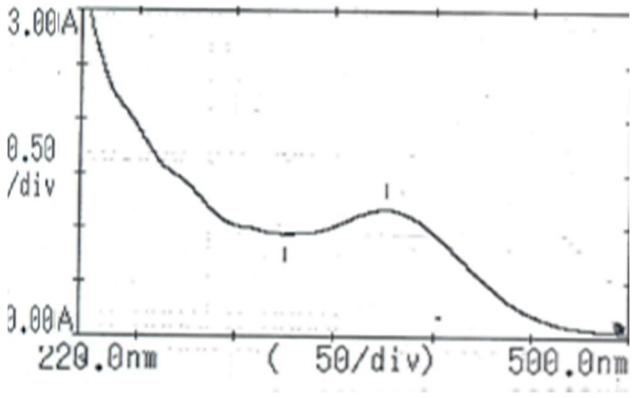


Fig. 2 : Sodium methoxide spectrum of compound I

Very significant structural features have been obtained by utilizing the so- called UV shift reagents which produce shifts in the UV absorption maxima in accordance with the location of the various hydroxyl functions in flavonoid nucleus ; these reagents are : sodium methoxide (which is diagnostic of 3- and 4`-OH functions);sodium acetate (diagnostic of 7-OH function); aluminium chloride (diagnostic of 3- , 5-OH and catechol systems) and boric acid (diagnostic of catechol systems).

When sodium acetate was added to a methanolic solution of compound II, no bathochromic shift diagnostic of a 7-OH function was observed(Fig.).

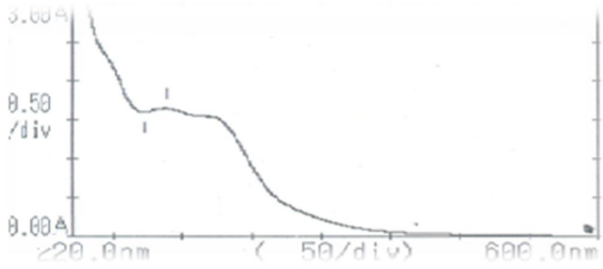


Fig. 3 : Sodium acetate spectrum of compound I

Other shift reagents -boric acid(Fig.4) , aluminium chloride (Fig,5)- failed to give any detectable bathochromic shifts. The boric acid spectrum thus suggests absence of catechols systems, while the aluminium chloride spectrum indicated absence of 3- and 5-hydroxylation as well as catechol moieties.

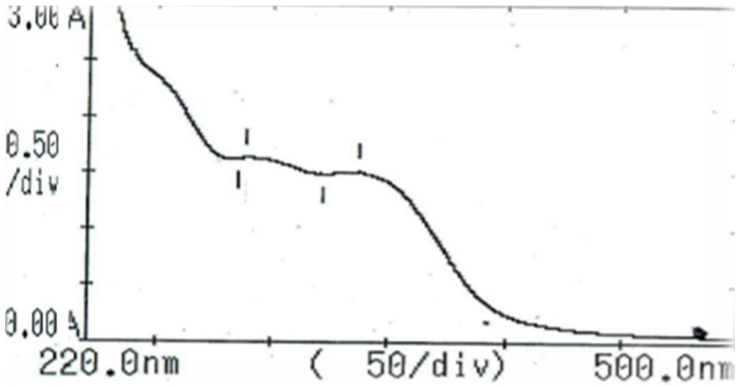


Fig. 4 : The boric acid spectrum of compound I

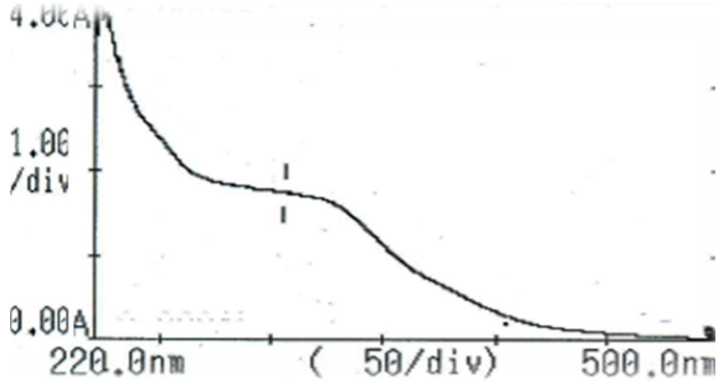


Fig. 5 : The aluminium chloride spectrum of compound I

The ^{1}HNMR spectrum (Figures 6_a and 6_b) showed: δ1.34(6H) assigned for 2 methyl functions; δ1.96(3H) assigned for an acetyl group ; δ3.40-4.00(m) attributed for sugar protons ; δ4.04 accounting for a methoxyl function . The aromatic protons appeared as multiplets centered at δ6.30 , δ6.80, δ7.65 and as singlet at δ7.10ppm.

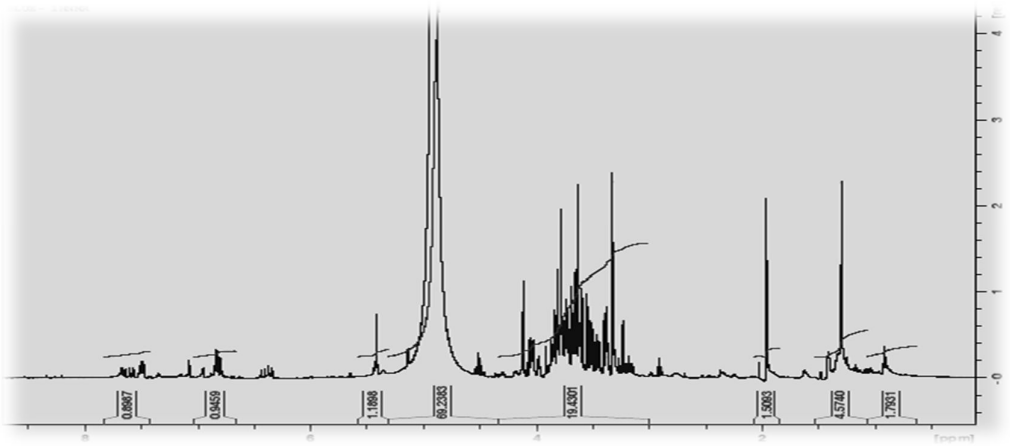


Fig.6_a : ^{1}HNMR spectrum of compound I

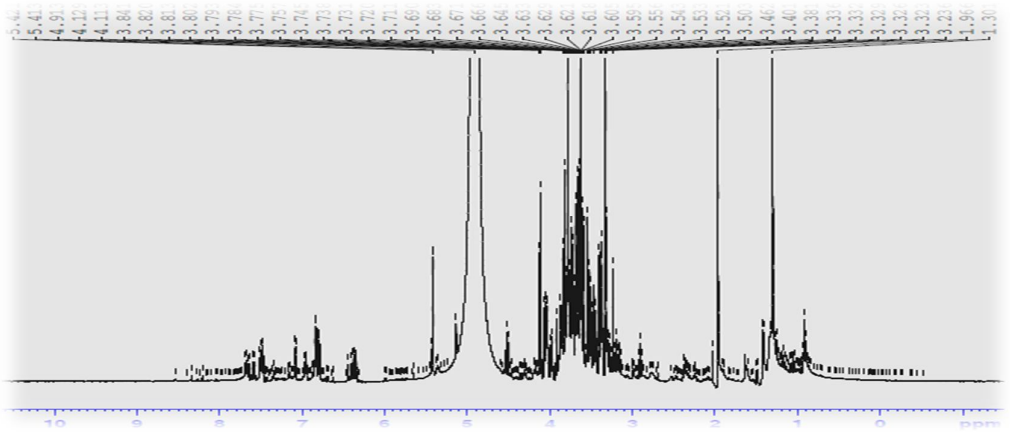
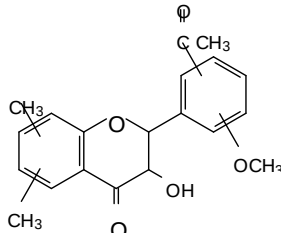


Fig.6_b : ^{1}HNMR spectrum of compound I

On the basis of the above cumulative data, the aglycone of compound I was assigned the following tentative structure:



I

The mass spectrum(Fig.7) gave m/z340 (for M^+ + H- aglycone) . Other fragments exceeding this vlaue are resulting from the glycoside whose sugar was not identified in this study

.

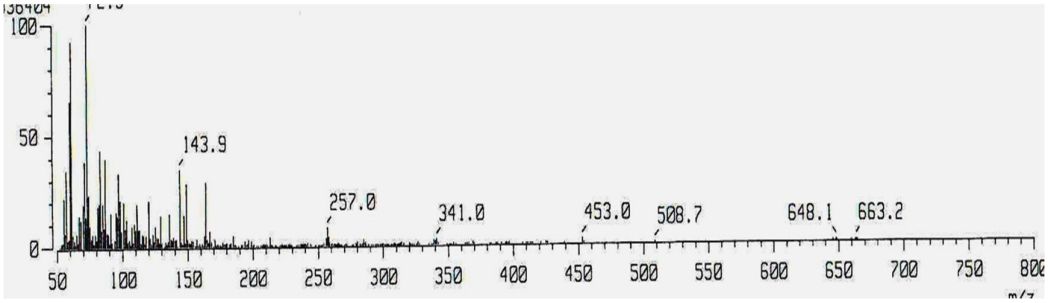
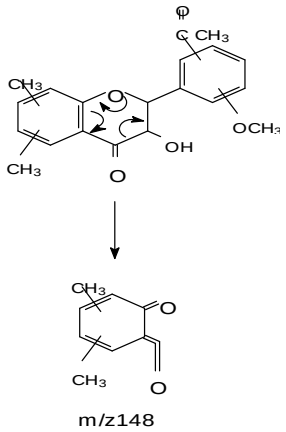


Fig. 7: Mass spectrum of compound I

The following retro Diels-Alder fission(Scheme I) suggests 2 methyl substituents for ring A:



Scheme I : Retro Diels-Alder fission of compound I (aglycone)

3.3-Characteization of compound II

In UV , compound II absorbs(Fig.8) at λ_{max} (MeOH) 265,298,312nm. Such absorption indicates conjugation between the 4- keto function

and the B aromatic ring of the flavonoid nucleus. This absorption is characteristic of flavones.

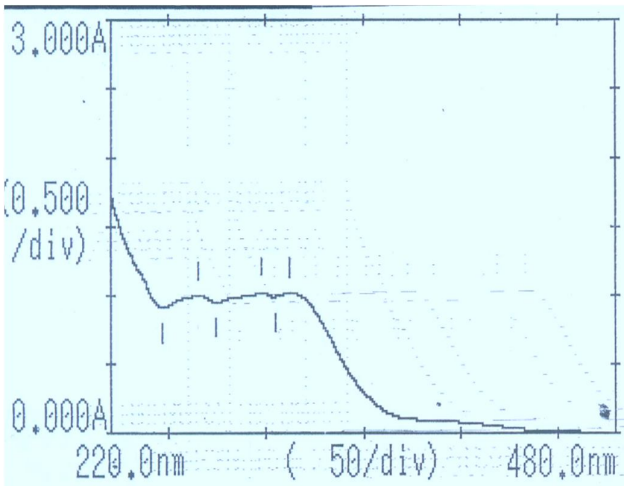


Fig.8 : UV spectrum of compound II

In their UV spectra flavones give both band I(due to cinnamoyl chromophore) and band II(due to benzoyl chromophore) , a feature which is shared by flavonols , chalcones and aurones[13] . Other classes: isoflavones, flavanones, dihydrochalcones and dihydroflavonols afford only one peak originating from the benzoyl system.

Very significant structural features have been obtained by utilizing the so- called UV shift reagents which produce shifts in the UV absorption maxima in accordance with the location of the various hydroxyl functions in flavonoid nucleus ; these reagents are : sodium methoxide (which is diagnostic of 3- and 4`-OH functions);sodium acetate (diagnostic of 7-OH function);aluminium chloride (

diagnostic of 3- , 5-OH and catechol systems) and boric acid (diagnostic of catechol systems).

The sodium methoxide spectrum (Fig.9) showed a bathochromic shift without decrease in intensity indicating a 4`-OH function.When sodium acetate was added to a methanolic solution of compound I, no bathochromic shift diagnostic of a 7-OH function was observed(Fig.10). Other shift reagents-boric acid(Fig. 11) , aluminium chloride (Fig.12)- failed to give any detectable bathochromic shifts. The boric acid spectrum thus suggests absence of catechols systems, while the aluminium chloride spectrum indicated absence of 3- and 5-hydroxylation as well as catechol moieties.

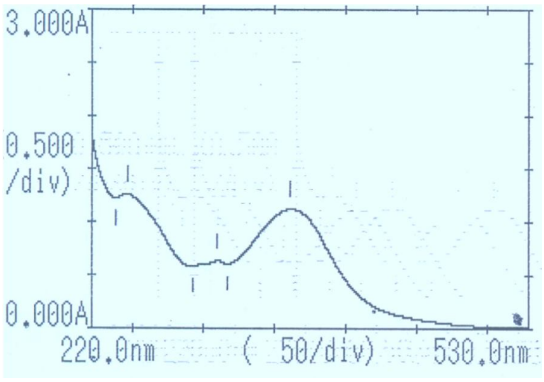


Fig. 9 : Sodium methoxide spectrum of compound II

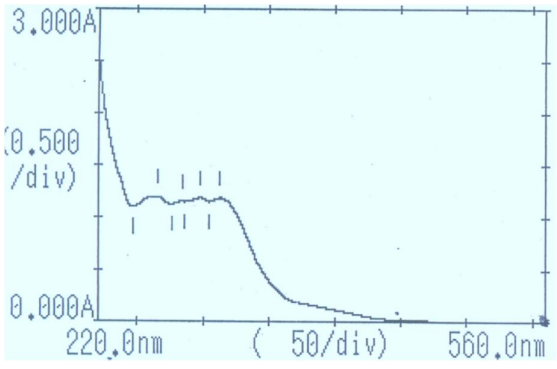


Fig. 10 : Sodium acetate spectrum of compound II

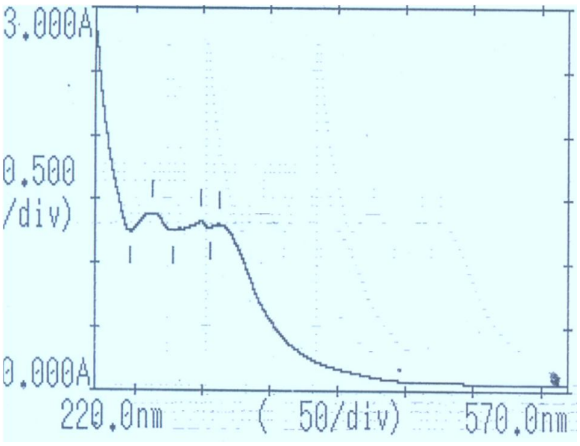


Fig. 11 : The boric acid spectrum of compound II

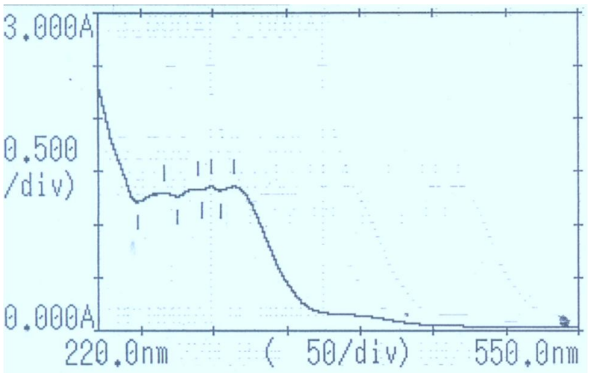


Fig. 12 : The aluminium chloride spectrum of compound II

The above UV data suggests that compound II is a flavone which is hydroxlated at the 4`-position.The ^{1}HNMR spectrum(Figures 13a and 13b) showed : δ1.34(12H) assigned for 4 methyl functions ; δ 1.90(3H) characteristic of an acetyl group ; δ3.55-3.83(m) assigned for a sugar moiety; δ6.36(1H) assigned for C_6- proton. Other aromatic protons appeared as a multiplet centered at δ7.80ppm. The EI mass spectrum gave m/z338 for (M^+ +2H – aglycone)

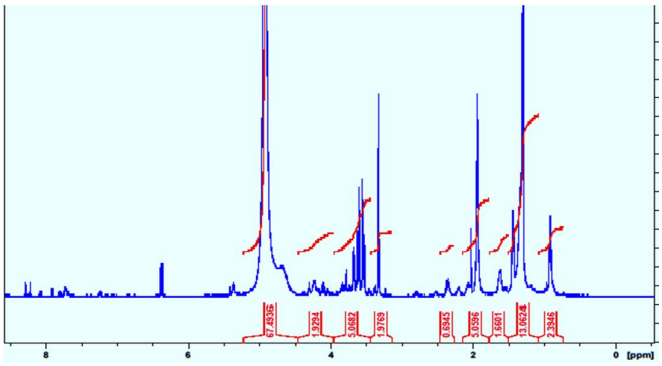


Fig.13a : ^{1}HNMR spectrum of compound I

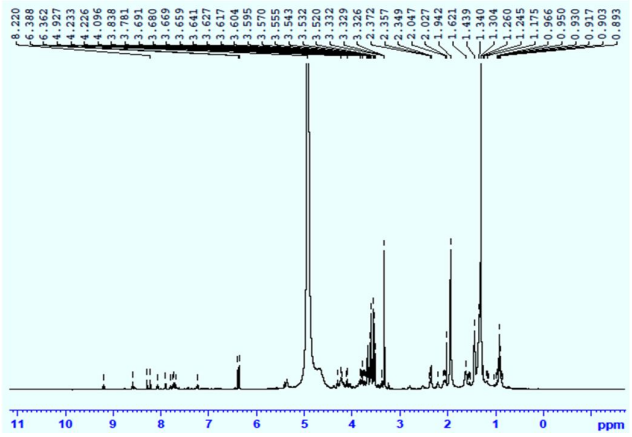
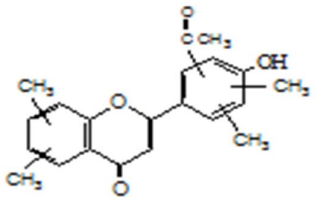


Fig.13b : ^{1}HNMR spectrum of compound I

On the basis of the above cumulative data , the following partial partial structure was proposed for the aglycone of compound II :



3.4-GC-MS analysis of *Trigonella foenum* fixed oil

Trigonella foenum oil was analyzed by GC-MS .MS library(NIST) was chcked for identification of the constituents. Furthermore , the observed fragmentation pattern was interpreted (MS library revealed about 90-95% match).

The GC-MS analysis showed the presence of 26 components(Table 5).The typical total ion chromatograms (TIC) is depicted in Fig.14.

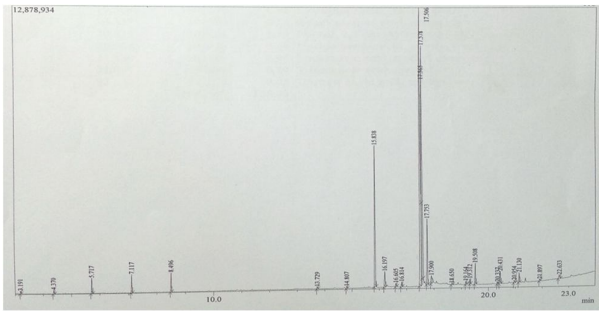


Fig.14: Total ion chromatograms

Table 5: Contituents of *Trigonella foenum* oil

Pk#	R.Time	Area	Area%	Name
1	3.191	159990	0.20	Styrene
2	4.370	278667	0.34	Octane, 3,5-dimethyl-
3	5.717	961604	1.18	Undecane
4	7.117	1206003	1.47	Dodecane
5	8.496	1265360	1.55	Tridecane
6	13.729	162751	0.20	Methyl tetradecanoate
7	14.807	154412	0.19	Pentadecanoic acid, methyl ester
8	15.838	10872233	13.29	Hexadecanoic acid, methyl ester
9	16.197	1457523	1.78	Pentadecanoic acid
10	16.605	284594	0.35	7-Hexadecenoic acid, methyl ester, (Z)-
11	16.814	375596	0.46	Heptadecanoic acid, methyl ester
12	17.506	29327852	35.85	9,12-Octadecadienoic acid (Z,Z)-, methyl e
13	17.565	8285287	10.13	9-Octadecenoic acid (Z)-, methyl ester
14	17.578	16086319	19.66	9,12,15-Octadecatrienoic acid, methyl este
15	17.753	5064721	6.19	Methyl stearate
16	17.900	712880	0.87	Oleic Acid
17	18.650	121001	0.15	Octadecanoic acid, 17-methyl-, methyl est
18	19.164	244733	0.30	Methyl 8,11,14-heptadecatrienoate
19	19.312	356302	0.44	cis-11-Eicosenoic acid, methyl ester
20	19.508	1585209	1.94	Eicosanoic acid, methyl ester
21	20.337	191320	0.23	Heneicosanoic acid, methyl ester
22	20.431	1005461	1.23	Phenol, 2,2'-methylenebis[6-(1,1-dimethyl
23	20.954	196923	0.24	13-Docosenoic acid, methyl ester, (Z)-
24	21.130	783109	0.96	Docosanoic acid, methyl ester
25	21.897	156486	0.19	Tricosanoic acid, methyl ester
26	22.633	511571	0.63	Tetracosanoic acid, methyl ester
		81807907	100.00	

Major constituents of the oil are discussed below:

Z,Z-9,12-Octadecadienoic acid methyl ester (35.85%)

Fig. 15 shows the EI mass spectrum of 9,12-octadecadienoic acid methyl ester.The peak at m/z294, which appeared at R.T. 17.506 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.

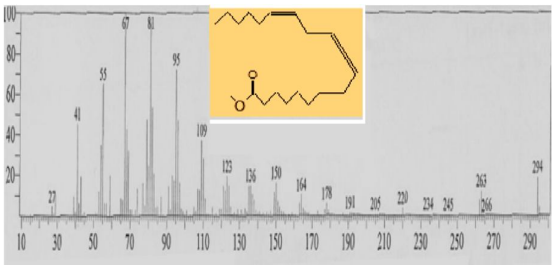


Fig. 15: Mass spectrum of 9,12-octadecadienoic acid methyl ester

9,12,15-Octadecatrienoic acid methyl ester(19.66%)

Mass spectrum of 9,12,15-octadecatrienoic acid methyl ester is depicted in Fig.16.The peak at m/z 292, which appeared at R.T. 17.578 corresponds $M^+[C_{19}H_{32}O_2]^+$ while the peak at m/z277 is attributed to loss of a methyl function.

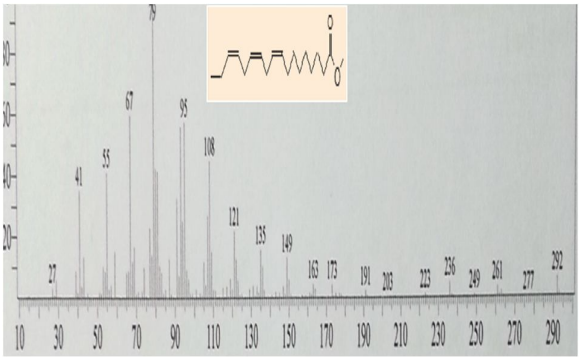


Fig. 16: Mass spectrum of 9,12,15-octadecatrienoic acid methyl ester

Hexadecanoic acid methyl ester(13.29%)

Mass spectrum of hexadecanoic acid methyl ester is depicted in Fig. 17.The peak at m/z 270, which appeared at R.T. 15.838 corresponds to $M^+[C_{17}H_{34}O_2]^+$ while the peak at m/z239 is attributed to loss of a methoxyl group.

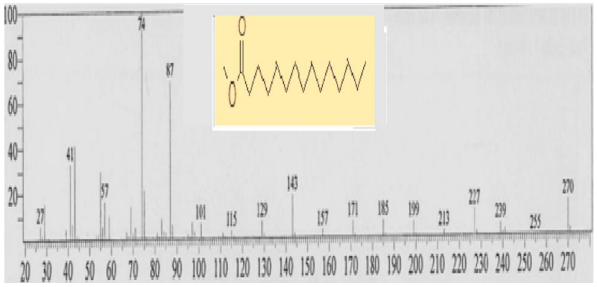


Fig. 17: Mass spectrum of hexadecanoic acid methyl ester

Z-9-Octadecenoic acid methyl ester(10.13%)

Fig. 18 shows the EI mass spectrum of 9-octadecenoic acid methyl ester.The peak at m/z 296, which appeared at R.T. 17.613 in total ion chromatogram, corresponds to $M^+[C_{19}H_{36}O_2]^+$, while the peak at m/z266 accounts for loss of a methoxyl function

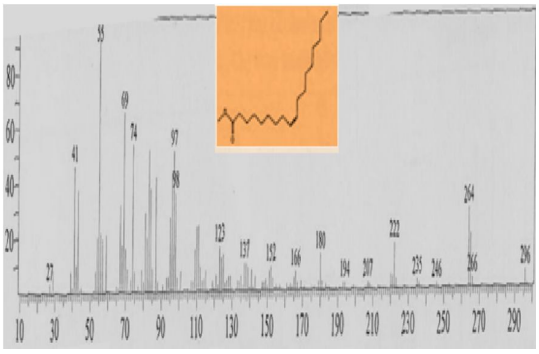


Fig. 18: Mass spectrum of 9-octadecenoic acid methyl ester

3.4.1-Antibacterial activity

Trigonella foenum fixed oil was screened for antimicrobial activity against five standard human pathogens. The results are depicted in Table (6) .The results were interpreted as follows : (<9mm: inative;9-12mm:partially active;13-18mm: active;>18mm:very active) .Tables (7) and (8) represent the antimicrobial activity of standard antibacterial and anifungal chemotherapeutic agents against standard bacteria and fungi respectively.

Table 6 : Antibacterial activity of *Trigonella foenum* oil

Type	Conc.(mg/ml)	**Sa**	Bs	Ec	Ps	Ca
Oil	100	20	14	15	15	17
	50	18	-	14	14	15
	25	17	-	13	13	10
	12.5	15	-	12	12	9
	6.25	11	-	10	7	-

Table 7 : Antibacterial activity of standard chemotherapeutic agents

Drug	Conc.(mg/ml)	Bs	Sa	Ec	Ps
Ampicilin	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 8 : Antifungal activity of standard chemotherapeutic agent

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

Sa.: *Staphylococcus aureus*
Ec.: *Escherichia coli*
Pa.: *Pseudomonas aeruginosa*
An.: *Aspergillus niger*
Ca.: *Candida albicans*
Bs.: *Bacillus subtilis*

The oil showed excellent activity against the bacterial strain *Staphylococcus aureus* in the concentration range : 100-25mg/ml. It also exhibited excellent activity against the yeast *Candida albicans* at 100mg/ml. The oil showed activity against all test organism at 100 mg/ml.

3.5-**GC-MS analysis of *Foeniculum vulgae* fixed oil**

GC-MS analysis of *Foeniculum vulgae* fixed oil was carried out. The MS library (NIST) was checked for identification of the constituents

(a 90-95% match was observed) . Furthermore, the observed fragmentation pattern was interpreted.

The GC-MS spectrum of the studied oil revealed the presence of 32 components(Table 9).The typical total ion chromatograms (TIC) is depicted in Fig.19.

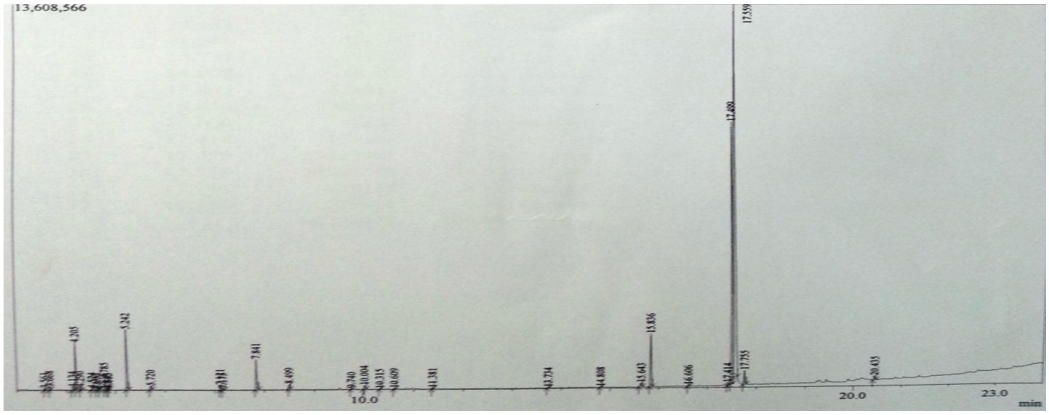


Fig.19: Total ion chromatograms

Table 9: Contituents of *Foeniculum vulgae* oil

Peak#	R.Time	Area	Area%	Name
1	3.561	78697	0.13	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-met
2	3.668	149117	0.25	.alpha.-Pinene
3	4.134	163802	0.27	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-me
4	4.205	2134541	3.51	.beta.-Pinene
5	4.290	123090	0.20	.beta.-Myrcene
6	4.524	62880	0.10	.alpha.-Phellandrene
7	4.603	16082	0.03	3-Carene
8	4.679	18436	0.03	(+)-4-Carene
9	4.785	512747	0.84	o-Cymene
10	4.843	48379	0.08	D-Limonene
11	4.865	42651	0.07	.beta.-Phellandrene
12	5.242	3027403	4.98	.gamma.-Terpinene
13	5.720	172319	0.28	Undecane
14	7.121	253023	0.42	Dodecane
15	7.175	86329	0.14	1-Cyclohexene-1-carboxaldehyde, 4-(1-me
16	7.841	1719357	2.83	Benzaldehyde, 4-(1-methylethyl)-
17	8.499	281517	0.46	Tridecane
18	9.740	54609	0.09	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-
19	10.004	210440	0.35	Benzenepropanol, 4-methoxy-
20	10.315	30972	0.05	Caryophyllene
21	10.609	70725	0.12	(E)-.beta.-Famesene
22	11.381	49224	0.08	Butylated Hydroxytoluene
23	13.734	37259	0.06	Methyl tetradecanoate
24	14.808	33780	0.06	Pentadecanoic acid, methyl ester
25	15.643	336214	0.55	9-Hexadecenoic acid, methyl ester, (Z)-
26	15.836	2902997	4.78	Hexadecanoic acid, methyl ester
27	16.606	76436	0.13	Methyl 8-heptadecenoate
28	17.414	159302	0.26	6,9-Octadecadienoic acid, methyl ester
29	17.499	17325881	28.50	9,12-Octadecadienoic acid (Z,Z)-, methyl e
30	17.559	29508900	48.54	9-Octadecenoic acid (Z)-, methyl ester
31	17.755	825729	1.36	Methyl stearate
32	20.435	279634	0.46	Phenol, 2,2'-methylenebis[6-(1,1-dimethyl
		60792472	100.00	

Main constituents of the oil are discussed below:

9-Z-Octadecenoic acid methyl ester(48.54%%)

Fig. 20 shows the EI mass spectrum of 9-octadecenoic acid methyl ester.The peak at m/z 296, which appeared at R.T. 17.559 in total ion chromatogram, corresponds to $M^{+}[C_{19}H_{36}O_2]^{+}$, while the peak at m/z266 accounts for loss of a methoxyl function

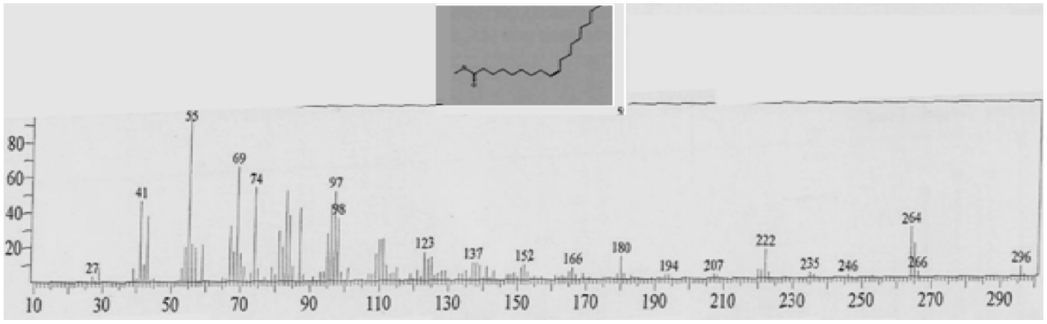


Fig. 20: Mass spectrum of 9-octadecenoic acid methyl ester

9,12-Z,Z-Octadecadienoic acid methyl ester (28.50%)

The mass spectrum of 9,12-octadecadienoic acid methyl ester is displayed in Fig.21.The peak at m/z294(R.T. 17.499-in total ion chromatogram)) corresponds $M^{+}[C_{19}H_{34}O_2]^{+}$ .The signal at m/z263 corresponds to loss of a methoxyl function.

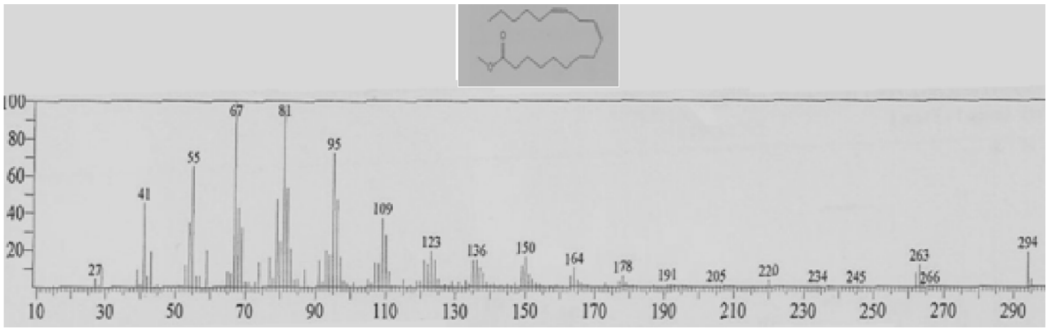


Fig. 21: Mass spectrum of 9,12-octadecadienoic acid methyl ester

γ – Terpinene(4.98%)

Fig. 22 shows the mass spectrum of γ – terpinene .The peak at m/z136 , which appeared at R.T. 17.559 in total ion chromatogram, corresponds $M^+[C_{10}H_{16}]^+$.

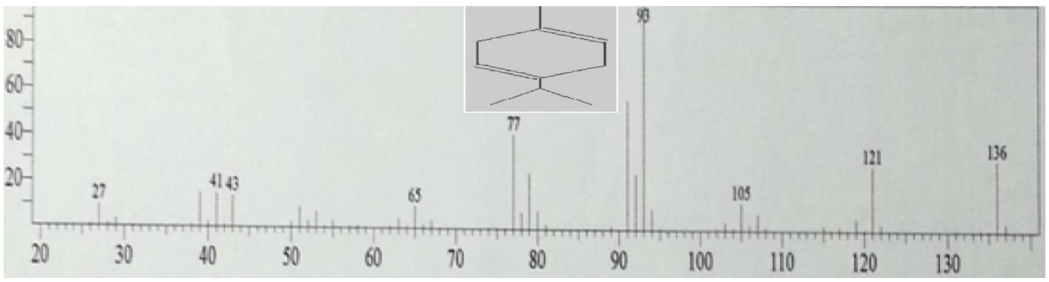


Fig. 22: Mass spectrum of γ – terpinene

β-Pinene(3.51%)

The masss spectrum of β-Pinene is shown in Fig.23. The molecular ion $M^+(C_{10}H_{16})$ appeared at m/z136 with RT,4.205 in total ion chromatogram.

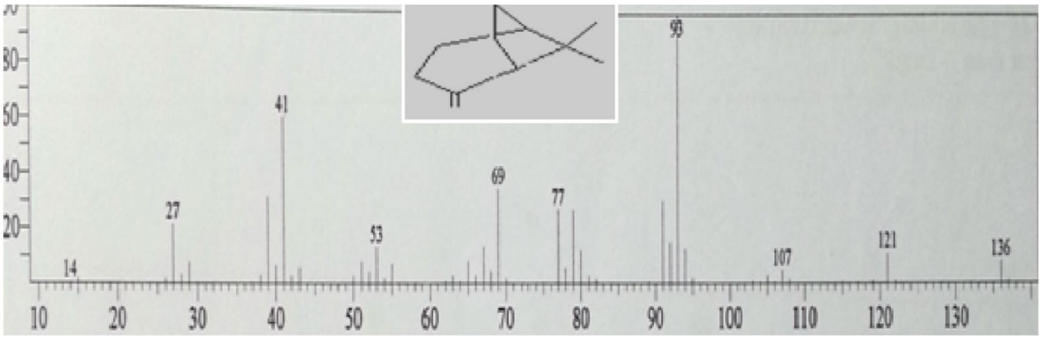


Fig. 23: Mass spectrum of β-pinene

3.5.1-Antibacterial activity

Foeniculum vulgae oil was screened for antimicrobial activity against five standard bacterial strains . The diameters of the growth of

inhibition zones are shown in Table (10) . Conventional terms were used for interpretation of the results : (<9mm: inative;9-12mm:partially active;13-18mm: active;>18mm:very active) .Tables (11) and (12) represent the antimicrobial activity of standard drugs..

Table 10 : Antibacterial activity of *Foeniculum vulgae* oil

Type	Conc.(mg/ml)	**Sa**	Bs	Ec	Ps	Ca
Oil	100	20	14	15	15	17
	50	18	-	14	14	15
	25	17	-	13	13	10
	12.5	15	-	12	12	9
	6.25	11	-	10	7	-

Table 11 : Antibacterial activity of standard chemotherapeutic agents

Drug	Conc.(mg/ml)	Bs	Sa	Ec	Ps
Ampicilin	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 12 : Antifungal activity of standard chemotherapeutic agent

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

Sa.: *Staphylococcus aureus*
Ec.: *Escherichia coli*
Pa.: *Pseudomonas aeruginosa*
An.: *Aspergillus niger*
Ca.: *Candida albicans*
Bs.: *Bacillus subtilis*

Foeniculum vulgae oil showed excellent activity against *Staphylococcus aureus* in the concentration range : 100-25mg/ml. It also exhibited significant activity against the yeast *Candida albicans* at 100mg/ml.

3.6-*Coriandrum Sativum*

The fixed oil of *Coriandrum Sativum* was extracted by maceration and analyzed by Gas Chromatography - Mass Spectrometry where 52 constituents were detected(Fig.24 and Table 13). Also antimicrobial activity was evaluated.

.

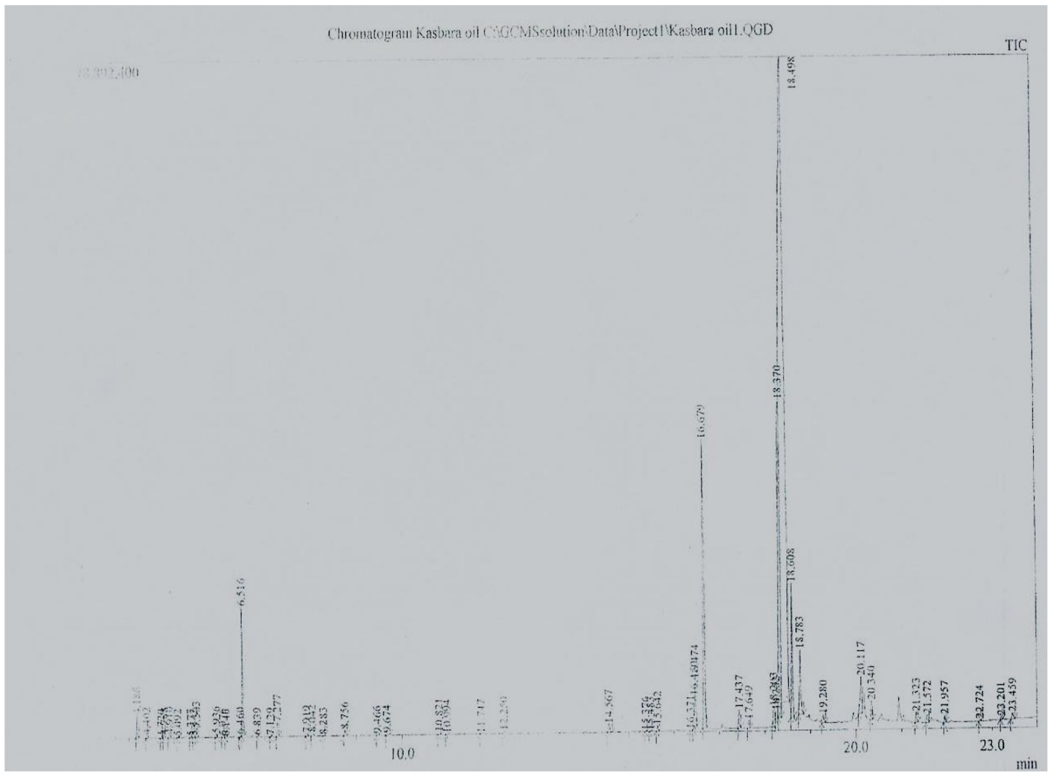


Fig.24: Total ions chromatogram

Major constituent of the oil are: 9-ocatadecanoic acid(Z)- methyl ester (61.60%). Then it is followed by 9,12-octadecadienioc acid (Z,Z)- methyl ester (14.15%), hexadecanoic acid methyl ester (8.04%), and methyl stearate (3.12%). The rest of the constituents have a percentage that dose not exceed (2.67%).

Table 13: Constituents of the oil

Peak Report FIC

Peak#	R.Time	Area	Area%	Name
1	4.186	3414165	0.41	.alpha.-Pinene
2	4.402	415376	0.05	Camphene
3	4.720	95322	0.01	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-me
4	4.788	327293	0.04	.beta.-Pinene
5	4.910	624749	0.07	.beta.-Myrcene
6	5.092	103650	0.01	Octanal
7	5.377	66410	0.01	Heptanoic acid, methyl ester
8	5.442	123215	0.01	p-Cymene
9	5.503	1401622	0.17	D-Limonene
10	5.936	470879	0.06	.gamma.-Terpinene
11	6.076	62489	0.01	1-Octanol
12	6.146	33725	0.00	.alpha.-Methyl-.alpha.-[4-methyl-3-penten
13	6.460	268865	0.03	Undecane
14	6.516	22273211	2.67	1,6-Octadien-3-ol, 3,7-dimethyl-
15	6.839	120323	0.01	Octanoic acid, methyl ester
16	7.129	104561	0.01	Methyl 1-cyclohexene-1-carboxylate
17	7.277	2442106	0.29	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethy
18	7.919	454710	0.05	L-.alpha.-Terpineol
19	8.042	520174	0.06	Decanal
20	8.283	125492	0.02	Nonanoic acid, methyl ester
21	8.756	1326269	0.16	Geraniol
22	9.466	187747	0.02	Undecanal
23	9.674	423212	0.05	Decanoic acid, methyl ester
24	10.821	415488	0.05	Dodecanal
25	10.994	102231	0.01	Undecanoic acid, methyl ester
26	11.747	88424	0.01	1,E-11,Z-13-Octadecatriene
27	12.250	484844	0.06	Dodecanoic acid, methyl ester
28	14.567	1670774	0.20	Methyl tetradecanoate
29	15.376	65185	0.01	5-Octadecenoic acid, methyl ester
30	15.483	110150	0.01	4-Octadecenoic acid, methyl ester
31	15.642	1088860	0.13	Pentadecanoic acid, methyl ester
32	16.371	226122	0.03	7,10-Hexadecadienoic acid, methyl ester
33	16.450	6455686	0.77	7-Hexadecenoic acid, methyl ester, (Z)-
34	16.474	7444450	0.89	9-Hexadecenoic acid, methyl ester, (Z)-
35	16.679	66947485	8.04	Hexadecanoic acid, methyl ester
36	17.437	3778590	0.45	14,17-Octadecadienoic acid, methyl ester
37	17.649	1553399	0.19	Heptadecanoic acid, methyl ester
38	18.203	6179904	0.74	6,9-Octadecadienoic acid, methyl ester
39	18.252	1760109	0.21	5,8-Octadecadienoic acid, methyl ester
40	18.370	117870779	14.15	9,12-Octadecadienoic acid (Z,Z)-, methyl e
41	18.498	513146985	61.60	9-Octadecenoic acid (Z)-, methyl ester
42	18.608	25988268	3.12	Methyl stearate
43	18.783	16807311	2.02	6-Octadecenoic acid, (Z)-
44	19.280	2514817	0.30	cis-10-Nonadecenoic acid, methyl ester
45	20.117	11137876	1.34	cis-11-Eicosenoic acid, methyl ester
46	20.340	3446015	0.41	Eicosanoic acid, methyl ester
47	21.323	2176449	0.26	Ethyl stearate, 9,12-diepoxy
48	21.572	2054112	0.25	9,12-Octadecadienoyl chloride, (Z,Z)-
49	21.957	1496863	0.18	Docosanoic acid, methyl ester
50	22.724	298620	0.04	Tricosanoic acid, methyl ester
51	23.201	640730	0.08	Heneicosane
52	23.459	1720021	0.21	Tetracosanoic acid, methyl ester
		833056112	100.00	

3.6.1- Antimicrobial activity

The total extract of *Coriandrum Stivum* seeds was evaluated for antimicrobial activity. The extract –unlike the oil which failed to exhibit any activity-gave significant activity against all test organisms(see Table 14). Noteworthy is the excellent anticandidial potency.

Table 14: The antimicrobial activity crude extract of *Coriandrum Stivum*

Organism	**Crude extract**
Bacillus subtitles	**20**
Staphylococcus aureus	**20**
Escherichia coli	**19**
Pseudomonas aeruginosa	**22**
Aspergillus niger	**18**
Condida albacans	**20**