3-Results and Discussion

The root of *Acacia nilotica* subspecies *tomentoza* were screened for major secondary metabolites. Phytochemical screening revealed the presence of flavonoids , tannins, steroids and alkaloids.

From roots a flavonoid was isolated by TLC in a chromatographically pure form. The structure of this flavonoid was elucidated by a combination of spectral tools (UV, ^{1}HNMR and MS). Furthemore, the total extract, chloroform and ethyl acetate fractions of roots were evaluated for their antimicrobial activity.

The oils from two key species in Sudanese ethnomedicine- *Cassia fistula* and *Eucalyptus camaldulensis* were analyzed by GC-MS and assessed for antimicrobial potential .

3.1- Identification of comound I

Compound I was isolated as yellow powder from the roots of *Acacia nilotica* by TLC. In the UV ,compound I absorbs at λ_{max} 282nm(Fig.1).

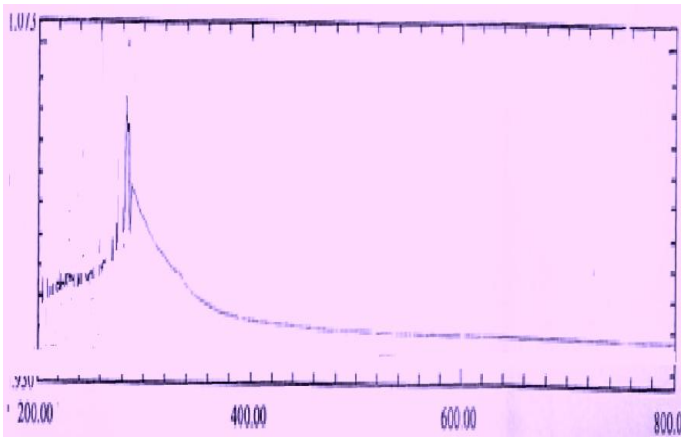
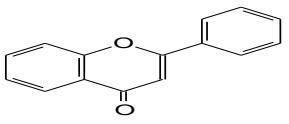


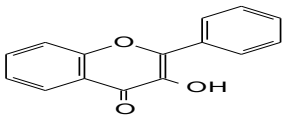
Fig. 1 : UV spectrum of compound I

The UV spectra of most flavonoids consists of two major absorption maxima, one of which occurs in the range 240-285 nm (band II) and the other in the range 300-400 nm (band I). In general terms the band II absorption may be considered as having originated from the A-ring benzoyl system and band I from the B-ring cinnamoyl system.

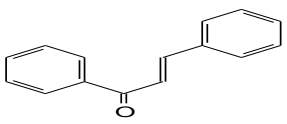
Flavonoids which afford two UV bands – due to conjugation between the carbonyl and the B ring are: flavones, flavonols , chalcones and aurones.



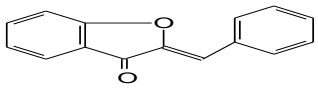
Flavone



Flavonol



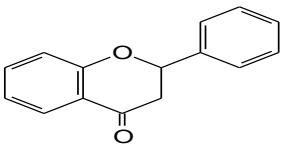
Chalcone



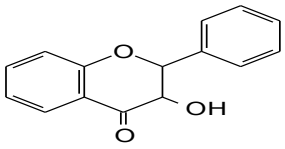
Aurone

Those flavonoids which lack conjugation between the carbonyl function and the B ring, namely: flavanones , isoflavones,

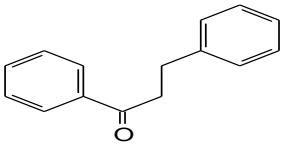
dihydroflavonols and dihydrochalcones eixhibit only one band – band II .



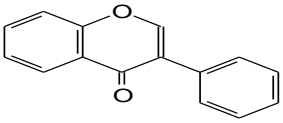
Flavanone



Dihydroflavonol



Dihydrochalcone



Isoflavone

Table 3.1: the UV absorption of some flavonoids

Flavonoid class	Band I	Band II
Flavones	304-350	240-280
Flavonol(3-OH substituted)	330-360	250-280
Flavonols(3-OH free)	350-390	250-280
Isoflavones	shoulder	245-270
Flavanones	shoulder	270-295
Dihdroflavonols	shoulder	270-295
Chalcones	365-390	240-260
Aurones	390-430	240-270

The UV absorption of compound I(282nm) is given by : flavanones, dihydroflavonols and isoflavones. However , no shoulder in the range 300-340nm (which is a characteristic feature of isoflavones) was detected in the spectrum(Fig.1).

The hydroxylation pattern in flavonoid nucleus is usually studied by using different UV shift reagents: sodium methoxide,sodium acetate, aluminium chloride and sodium acetate/boric acid. Such reagents induce bathochromic shifts diagnostic of certain hydroxylation pattern. Sodium methoxide is diagnostic of 3- and 5-OH functions, while sodium acetate is diagnostic of 7-hydroxylation pattern. The shift reagent aluminium chloride is diagnostic of 3- , 5-OH as well as catechol systems.

The sodium methoxide spectrum (Fig.2) did not show any bathochromic shift indicating absence of 3- and 4`-OH functions. However , the sodium acetate spectrum(Fig.3) gave a bathochromic shift diagnostic of a 7-OH group.

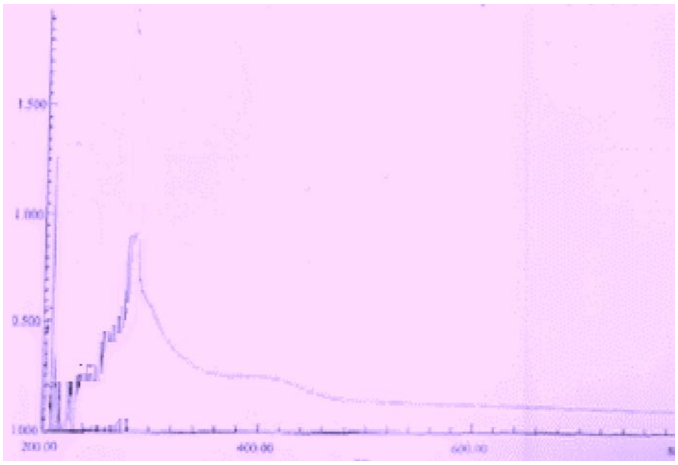


Fig. 2 : Sodium methoxide spectrum of compound I

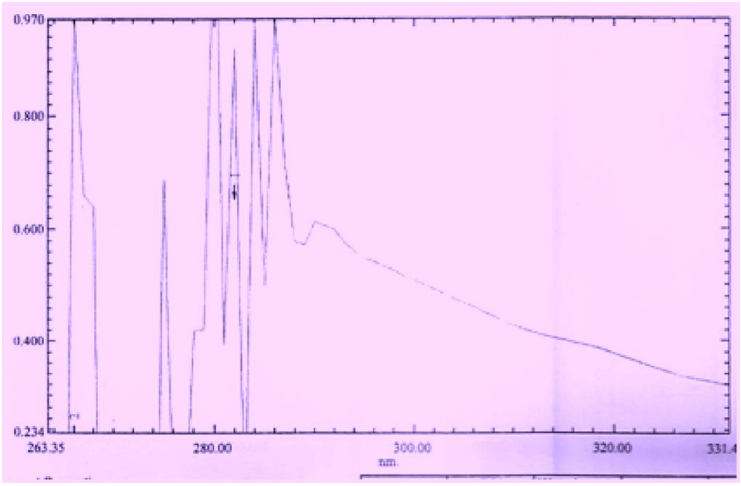
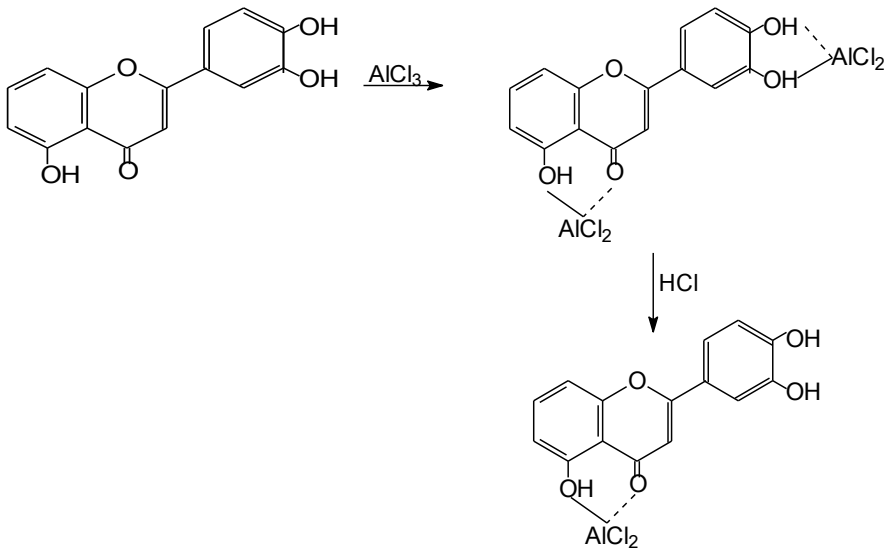


Fig. 3 : Sodium acetate spectrum of compound I

Aluminium chloride chelates with functional groups such as: the 5-hydroxy-4-keto-, 3-hydroxy-4-keto-and o-dihydroxy1 systems, and this reaction is evidenced by bathochromic shifts of one or both bands in the spectrum.Unlike the catechol complex, the 3- and 5-OH complexes are acid-stable as shown below:



The aluminium chloride spectrum of compound I(Fig. 4) did not reveal a bathochroic shift . This lends evidence for absence of 3- and 5-OH functions as well as catechol moieties. The boric

acid spectrum also showed absenc of catechol , since it did not reveal anybathochromic shift(Fig. 5).

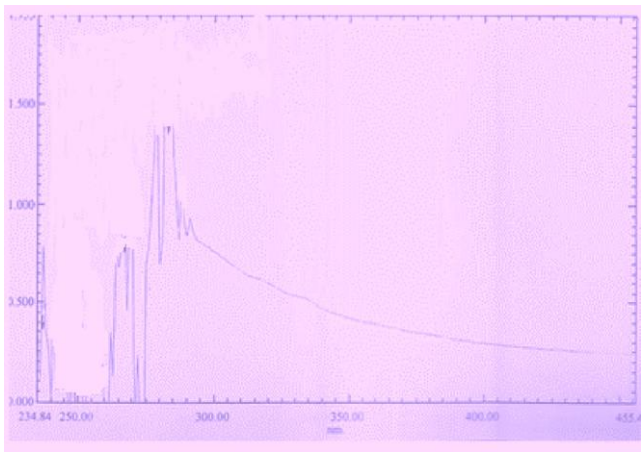


Fig. 4 : Aluminium chloride spectrum of compound I

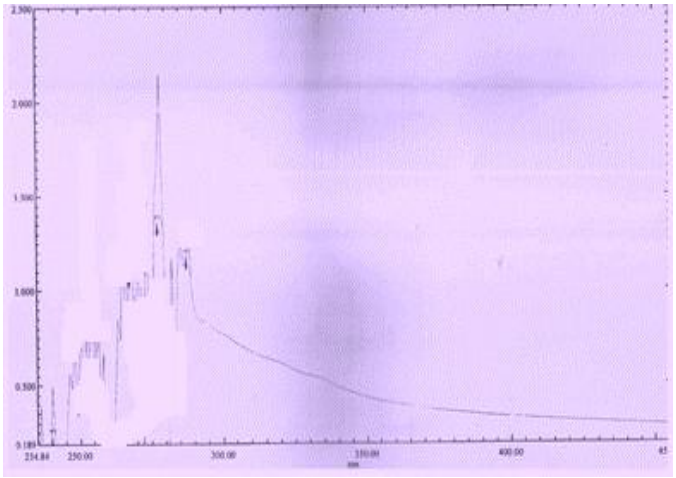


Fig. 5 : Boric acid spectrum of compound I

The isolated flavonoid is not an isoflavone as indicated above. Also , the absence of a 3-OH (sodium methoxide spectrum) excludes dihydroflavonols. It could be a flavanone or a dihydrochalcone. Flavanones and dihydrochalcones are usually distinguished by their ^{1}HNMR spectra. Flavanones show two multiplets around δ2.80 and δ5.20ppm due to mutual splitting of the magnetically unequivalent protons at C_3 .Such splitting gives

a pair of doublets which are further splited by C_2 proton into a double doublet(usually overlapping into a double multiplet).

The ^{1}HNMR (Fig.6)showed : δ1.12(3H) assigned for a methyl group; δ1.77(6H) accounting for two acetyl functions; δ3.52(3H) attributed to a methoxyl group. The multiplet at δ3.58-4.15 is due to a sugar moiety. The aromatic protons resonated at δ6.85, δ7.40 and δ8.36.

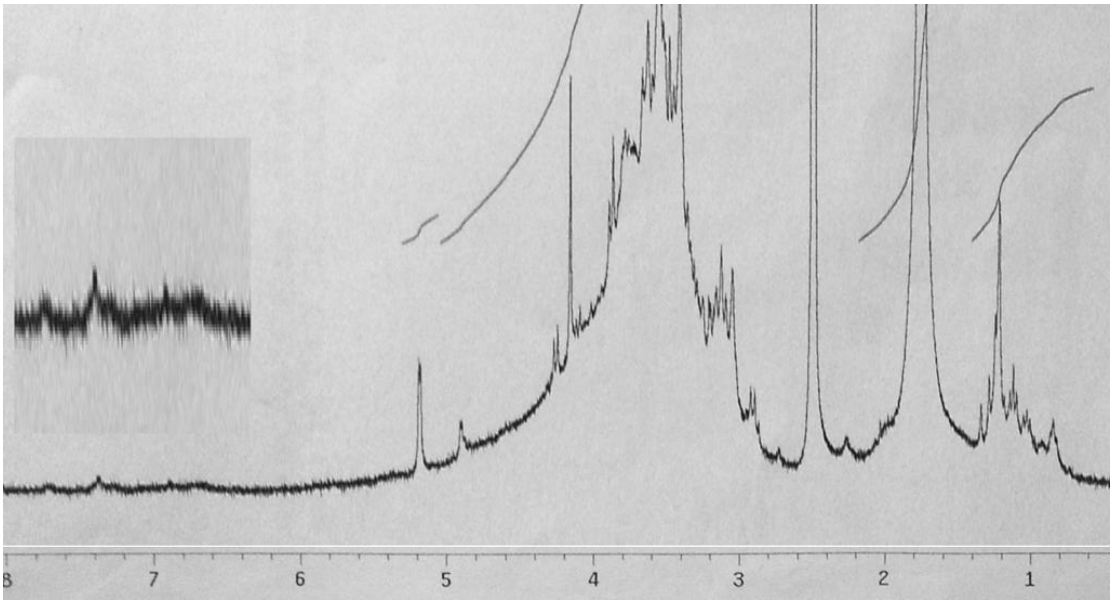


Fig.6 : ^{1}HNMR spectrum of compound I

No multiplets at δ2.80 and δ5.20 characteristic of flavanones was observed in the spectrum. This indicates that the isolated flavonoid is a dihydrochalcone.The mass spectrum (Fig.7) gave m/z339 for M^+ - H .

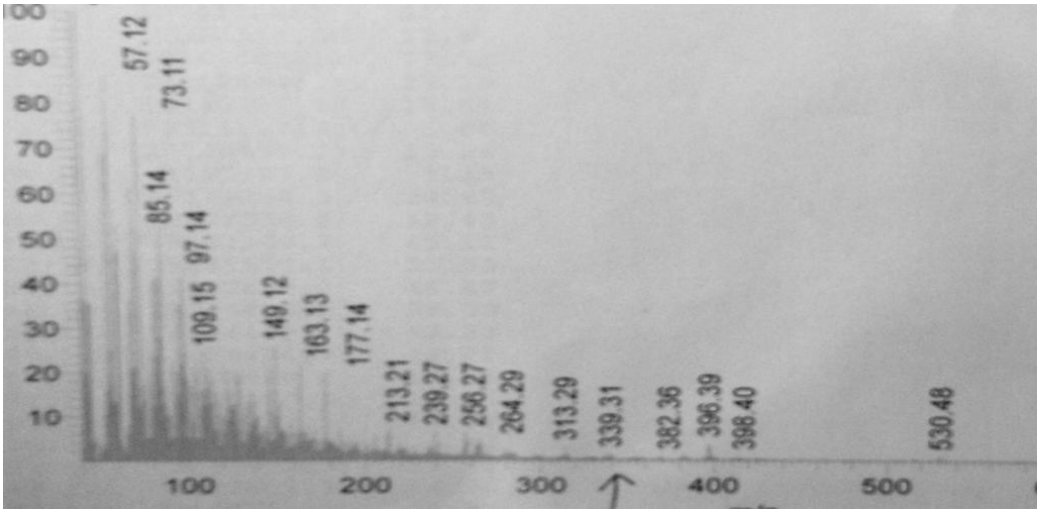
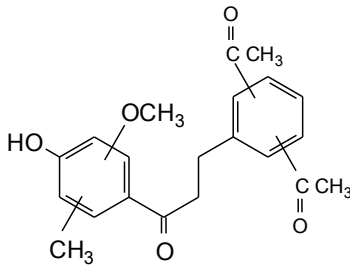


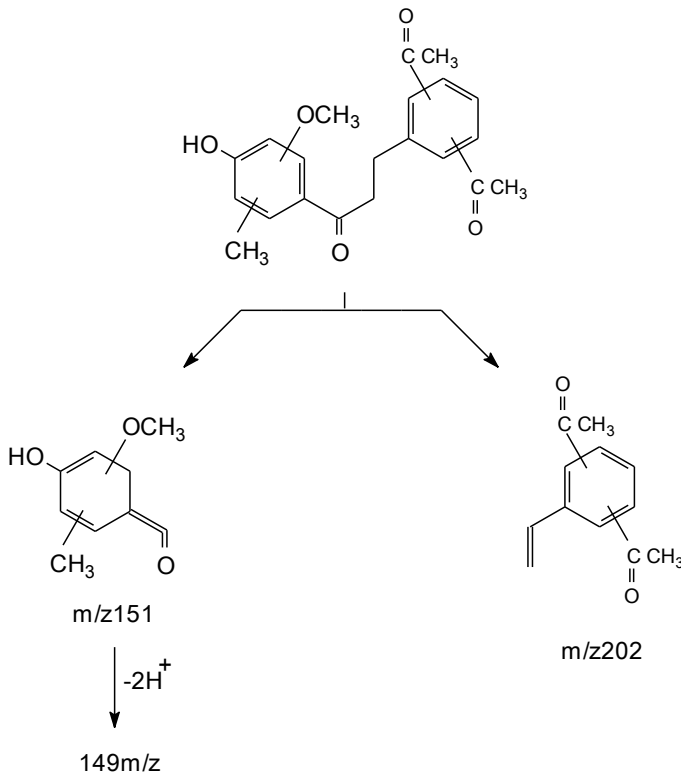
Fig.7 :Mass spectrum of compound I

On the basis of the above spectral data the following partial structure was assigned for compound I :



I

The retro Diels – Alder fission (Scheme I) lends additional evidence in favor of the proposed structure.



Scheme I:Retro Diels-Alder fission

3.1.1-Antimicrobial activity

The total extract, chloroform and ethyl acetate fractions of *Acacia nilotica* roots were evaluated for their antimicrobial activity against five standard microbial strains : *Staphylococcus aureus* , *Escherichia coli* , *Pseudomonas aeruginosa* , *Bacillus subtilis* and *Candida albicans.*

The diameters of the growth of inhibition zones are shown in Table (3.2) . Conventional terms were used for interpretation of the results : (<9mm: inative;9-12mm:partially active;13-18mm: active;>18mm:very active) .Tables (3.3) and (3.4) represent the antimicrobial activity of standard drugs..

Table 3.2 : Antibacterial activity of *Acacia nilotic root extracts*

Type	Conc.(mg/ml)	**Sa**	Bs	Ec	Ps	Ca
Ethanol extract	100	16	16	18	--	--
	50	14	14	15	--	--
	25	12	13	15	--	--
	12.5	12	--	12	--	--
	6.25	--	--	12	--	--
Chloroform extract	100	--	--	10	--	--
Ethyl acetate extract	100	17	17	13	--	--
	50	15	17	11	--	--
	25	10	13	10	--	--
	12.5	--	13	--	--	--
	6.25	--	--	--	--	--

Table 3.3 : 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 3.4 : 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 ethanol extract showed excellent activity against *Escherchia coli*, *Staphylococcus aureus* and *Bacillus subtilis* at a dose of 100mg/ml. At 50 mg/ml it was also active against *Escherchia coli*, *Staphylococcus aureus* and *Bacillus subtilis* . Excellent activity against *Bacillus subtilis* and *Staphylococcus aureus* has been exhibited by ethyl acetate fraction at a dose of 100mg/ml. However, the chloroform fraction failed to give any activity against all test organism at test concentrations.

3.2- *Eucalyptus camaldulensis* oil

3.2.1-GC-MS analysis of oil

GC-MS analysis of *Eucalyptus camaldulensis* fixed oil was carried out. The MS library (NIST) was checked for identification of the constituents (a 90-95% match was observed) . Furthermore, the resulting fragmentation pattern was discussed. 29 components were detected by GC-MS anslysis(Table 3.5).The typical total ion chromatogram (TIC) is depicted in Fig.(8).

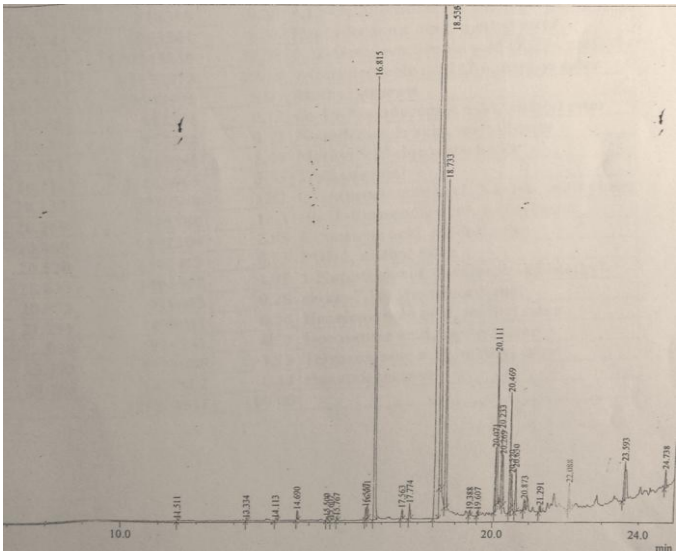
o 

Fig.8: Total ion chromatograms

Table 3.5: Contituents of *Eucalyptus camaldulensis*

No.	R. Time	Area%	Name
1	11.511	0.04	Alloaroanadendrene
2	.33413	0.05	Cyclheptane,4-methylene-1-methyl
3	14.113	0.07	2-Naphthalenemethanol,decahydro-alpha-
4	14.690	0.20	Methyl tetrdecanoate
5	15.500	0.09	Cis-5-Dodecenoic acid methyl ester
6	15.607	0.04	5-Octadeanoic acid,methyl ester
7	15.767	0.09	Pentadecanoic acid, methyl ester
8	16.557	0.32	7-Hexadecanoic acid,methyl ester
9	16.601	0.30	9-Hexadcanoic acid, methyl ester
10	16.815	14.99	Hexadecanoic acid,methyl ester
11	17.563	0.25	9,12-Octadecadienoyl chloride
12	17.774	0.35	Heptadecanoic acid,methyl ester
13	18.536	39.88	9,12-Octadecadienoic acid methy ester(Z,Z-)
14	18.553	19.10	9-Octadecenoic acid methyl ester(z)
15	18.733	9.06	Methyl stearate
16	19.388	0.13	Cis-10-Nonadecesoic acid,methyl ester
17	19.607	0.12	Nonadecanoic acid, methyl ester
18	20.071	1.48	Methyl-5,13-docosadienoate
19	20.111	3.38	Tridecanedial
20	20.233	1.82	Oxiraacoctanoic acid,3-octyl-methyl ester
21	20.269	1.03	Cis-11-Eicosenoic acid,methyl

			ester
22	20.469	2.85	Eicosanoic acid,methyl este
23	20.520	0.73	PGHI,methyl ester
24	20.630	1.05	1-Naphthalenol dechydro-4a-,methyl ester
25	20.873	0.28	Cis,cis,7,10-Hexedecdienal
26	21.291	0.14	Heneicosanoic acid , methyl ester
27	22.088	0.59	Docosanoic acid,methyl ester
28	23.593	0.15	Tetracosanoic acid , methyl ester
29	24.738	0.44	Hexatriacontane
		100%	

Main constituents of the oil are discussed below:

9,12-Z,Z-Octadecadienoic acid methyl ester (39.88%)

The mass spectrum of 9,12-octadecadienoic acid methyl ester is displayed in Fig.9.The peak at m/z294(R.T. 18.536 -in total ion chromatogram) corresponds $M^+[C_{19}H_{34}O_2]^+$ .The signal at m/z263 corresponds methoxyl function.

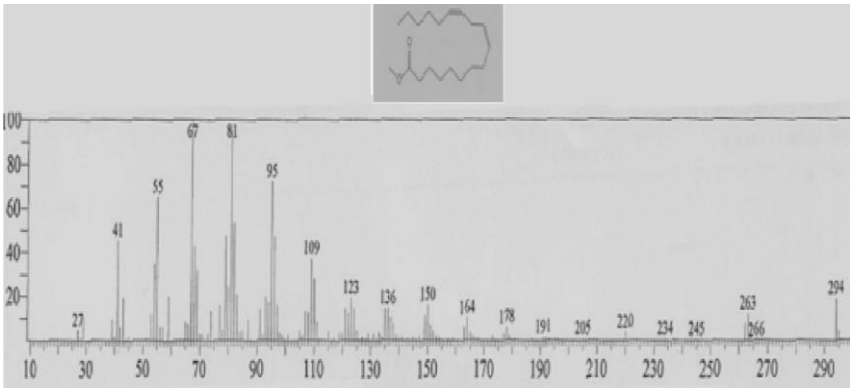


Fig. 9: Mass spectrum of 9,12-octadecadienoic acid methyl ester

9-Z-Octadecenoic acid methyl ester(19.10%%)

Fig. 10 shows the EI mass spectrum of 9-octadecenoic acid methyl ester.The peak at m/z 296, which appeared at R.T. 18.553 in total ion chromatogram, corresponds $M^+[C_{19}H_{36}O_2]^+$, while the peak at m/z266 accounts for loss of a methoxyl .

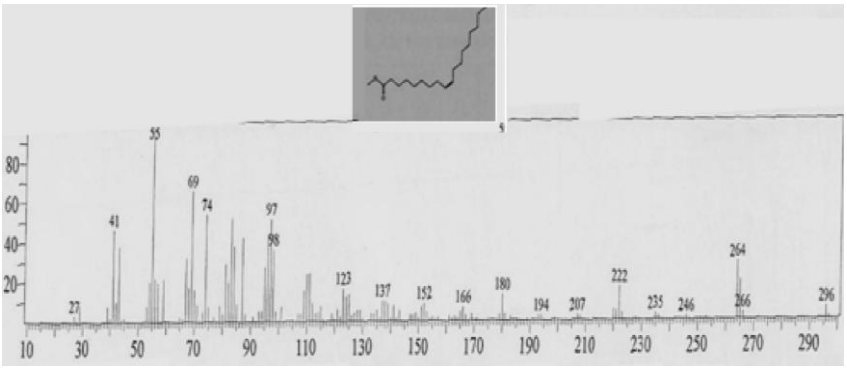


Fig. 10: Mass spectrum of 9-octadecenoic acid methyl ester

Hexadecanoic acid methyl ester(14.99%)

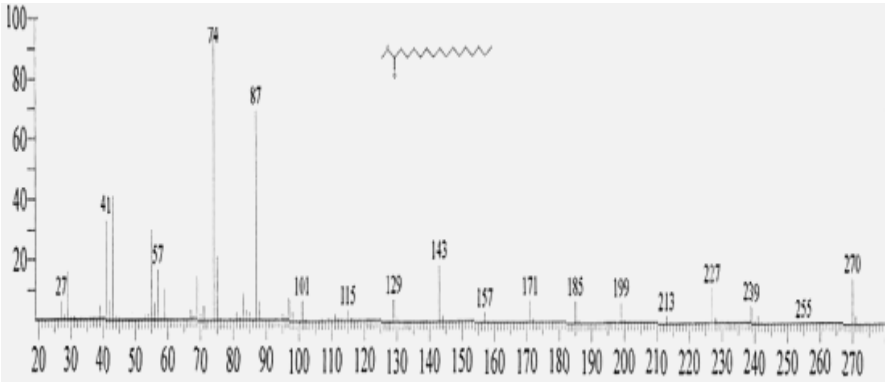


Fig. 11: Mass spectrum of hexadecanoic acid methyl ester

The mass spectrum of hexadecanoic acid methyl ester is depicted in Fig.11.The peak at m/z 270 (R.T.16.815) corresponds $M^{+}[C_{17}H_{34}O_2]^{+}$ .The signal at m/z239 corresponds to loss of a methoxyl**.**

Methyl stearate(9.06%)

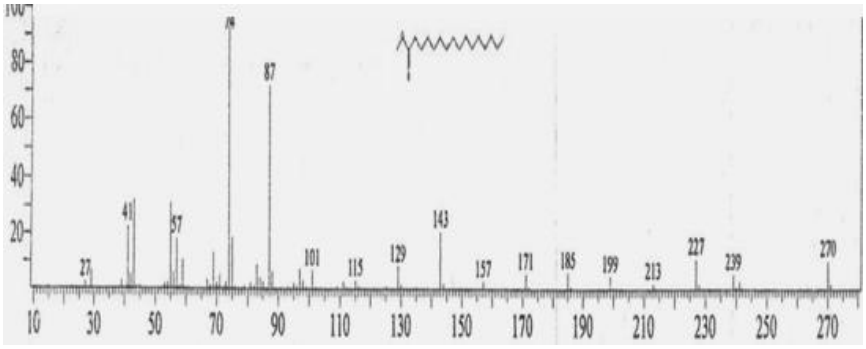


Fig. 12: Mass spectrum of methyl stearate

Fig. 12 shows the mass spectrum of methyl stearate .The signal at m/z 298(R.T.18.733) corresponds $M^{+}[C_{19}H_{38}O_2]^{+}$, while the peak at m/z267 corresponds to loss of a methoxyl group.

Tridecanedial(3.38%)

The mass spectrum of tridecanedial is depicted in Fig.13.The peak at m/z 212 (R.T. 20.111) corresponds $M^{+}[C_{13}H_{24}O_2]^{+}$.

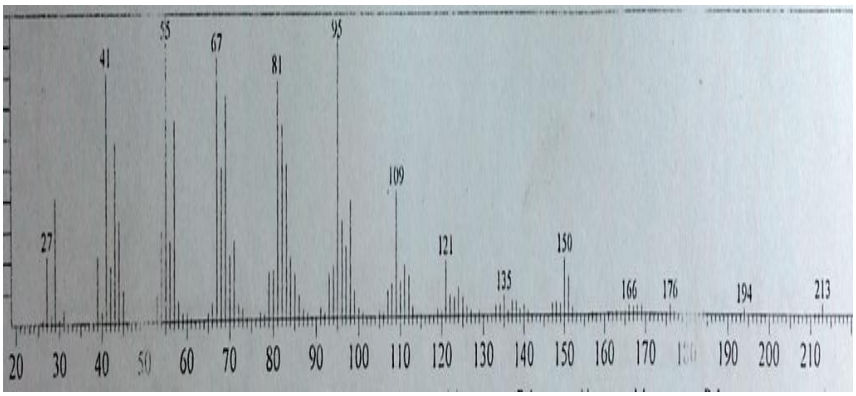


Fig. 13: Mass spectrum of tridecanedial

Antibacterial activity

Eucalyptus camaldulensis oil was screened for antimicrobial activity against five standard bacterial strains . The diameters of the growth of inhibition zones are shown in Table (3.6) . Conventional terms were used for interpretation of the results : (<9mm: inative;9-12mm:partially active;13-18mm: active;>18mm:very active) .

Table 3.6 : Antibacterial activity of *Eucalyptus camaldulensis* oil

Type	Conc.(mg/ ml)	**Sa**	Bs	Ec	Ps	Ca
Oil	100	20	--	19	19	20
	50	19	-	18	18	19
	25	18	-	17	17	18
	12.5	18	-	13	16	18
	6.25	15	-	12	13	17

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

The oil showed excellent activity against all test microorganisms-except for *Bacillus subtilis* - in the concentration range : 100-25mg/ml.It also exhibited significant activity against the yeast *Candida albicans* at 12.5 and 6.25mg/ml.

3.3-*Cassia fistula* oil

3.3.1-GC-MS analysis of the oil

GC-MS analysis of *Cassia fistula* oil was conducted and the identification of the constituents was initially accomplished by comparison with the MS library (NIST).The GC-MS analysis of the studied oil revealed the presence of 25 components(see Table 3.7 and Fig.14).

Table 3.7: Constiuents of *Cassia fistula* oil

No.	R.Time	Area%	Name
1	5.349	0.05	1-Octanol
2	7.154	0.07	L-α-Terpineol
3	8.858	0.01	Decanoic acid, methyl ester
4	11.427	0.04	Dodecanoic acid methyl ester
5	13.751	0.92	Methyl tetracecanoate
6	14.563	0.11	5-Octadecenoic acid , methyl ester
7	14.829	0.31	Pentadecanoic acid, methyl ester
8	15.665	0.85	9-Hexadecanoic acid, methyl ester
9	15.874	13.55	Hexadecanoic acid, methyl ester
10	16.628	0.27	Cis-10-Heptadecenoic acid, methyl ester
11	16.671	0.09	6-Octadecenoic acid, methyl ester
12	16840	0.45	Heptadecanoic acid, methyl ester
13	17.543	24.46	9,12-Octadecadienoic acid, methyl ester
14	17.596	17.26	9-Octadecenoic acid , methyl ester
15	17.786	7.75	Methyl stearate
16	19.178	2.25	9,12-Octadecadienoyl chloride,z,z-
17	19.305	0.92	Oxiraneoctanoic acid,3-octyl-, methyl ester
18	19.339	1.02	11-Eicosenoic acid, methyl ester
19	19.539	3.72	Eicosanoic acid, methyl ester
20	19.710	0.54	Methyl 15-hydroxy-9,12-octadecadienoate
21	20.623	15.75	Stigma-7-en-3-ol,(3β-5-α-24S)
22	21.163	3.63	Docosanoic acid, methyl ester
23	21.929	0.92	Tricosanoic acid, methyl ester
24	22.667	3.63	Tetracosanoic acid, methyl ester
25	23.384	1.42	Methyl 22-methyltetracosanoate
		100.00	

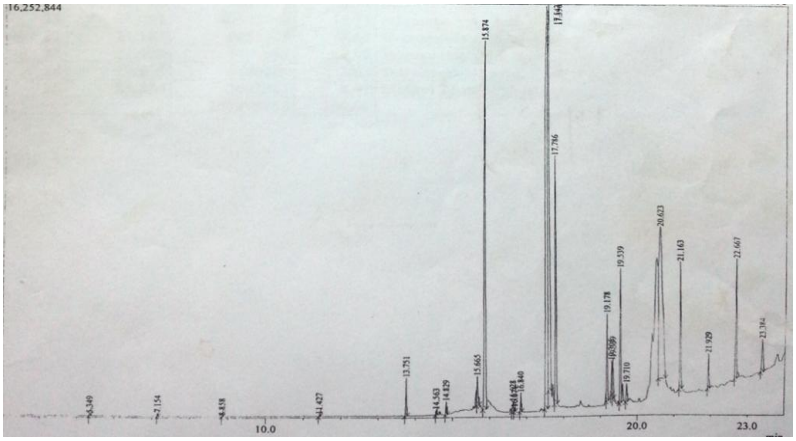


Fig.14: Total ions chromatogramsof the oil

Some important constituents are discussed below:

9,12-Octadecadienoic acid methyl ester (24.46%)

Fig. 15 shows the EI mass spectrum of 9,12-octadecadienoic acid methyl ester.The peak at m/z294, which appeared at R.T. 17.543 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.

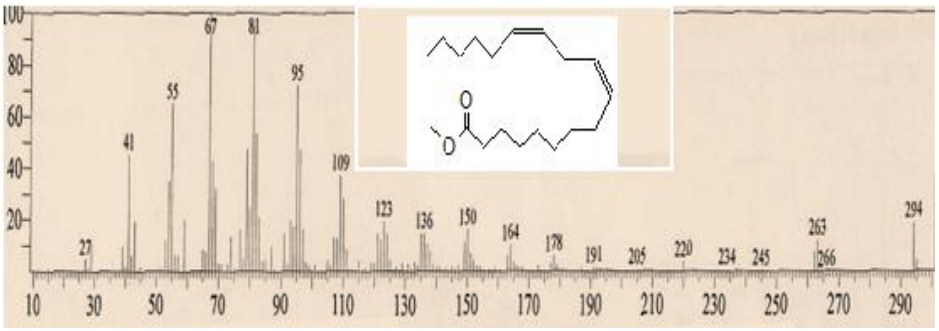


Fig. 15: Mass spectrum of 9,12-octadecadienoic acid methyl ester

Linoleic acid (9,12-octadecadienoic acid) can not be synthesized by humans and is available through diet.It belongs to one of the two families of essential fatty acids. It exists in lipids of cell membrane and is used in the biosynthesis of arachidonic acid . It is converted enzymatically into mono-hydroxy products which are subsequently oxidized by some enzymes to keto metabolites. These metabolites are implicated in human physiology and pathology. Deficiency of linolate caused hair loss and poor wound healing in model animals.

9-Octadecenoic acid methyl ester(17.26%)

The mass spectrum of 9-octadecenoic acid methyl ester is displayed in Fig.16.The peak at m/z 296, which appeared at R.T. 17.596 in total ion chromatogram, corresponds $M^+[C_{19}H_{36}O_2]^+$, while the peak at m/z266 accounts for loss of a methoxyl .

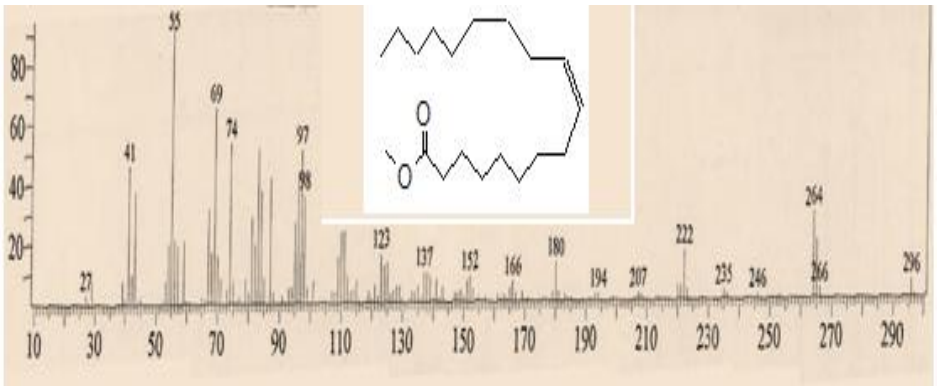


Fig. 16: Mass spectrum of 9-octadecenoic acid methyl ester

Oleic acid (9-octadecenoic acid) acid is a common monounsaturated fat in human diet. It may be responsible for the hypotensive potential of olive oil. Oleic acid finds some applications in soap industry and it is used in small amounts as excipient in pharmaceutical industries. It is employed as emollient. The consumption of oleate in olive oil has been associated with decreased risk of breast cancer.

Stigmast-7-en-3-ol(15.75%)

The mass spectrum of stigmast-7-en-3-ol is depicted in Fig. 17.The peak at m/z 414 (R.T. 20.623) corresponds

$M^+[C_{29}H_{50}O]^+$ while the signal at m/z399 is attributed to loss of a methyl group.

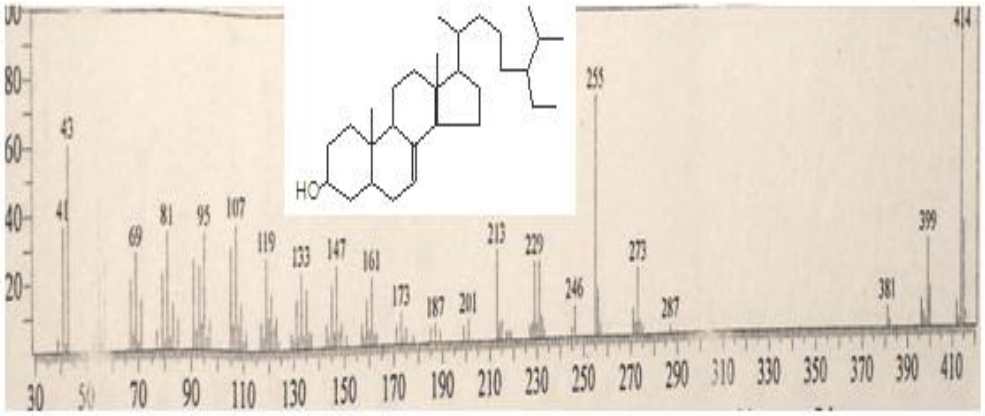


Fig. 17: Mass spectrum stigmast-7-en-3-ol

Hexadecanoic acid methyl ester(13.55%)

Fig. 18 shows the mass spectrum of hexadecanoic acid methyl ester .The peak at m/z 270 (R.T. 15.874) corresponds $M^+[C_{17}H_{34}O_2]^+$ while the signal at m/z239 is due to loss of a methoxyl function.

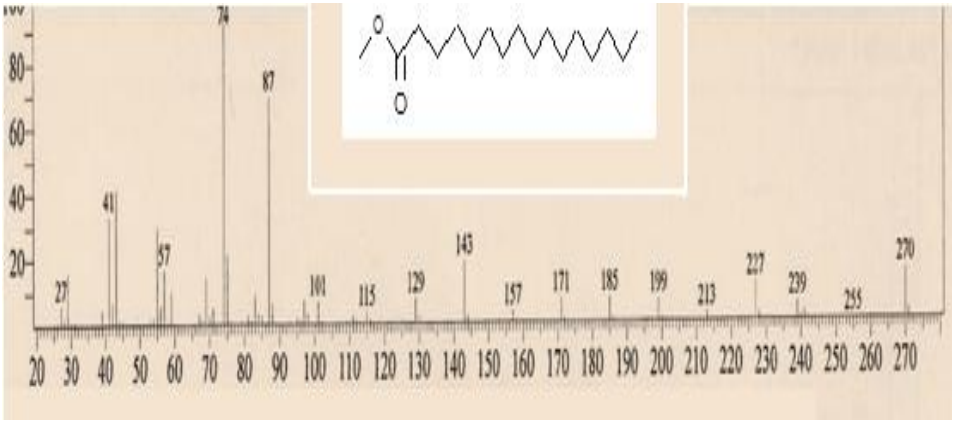


Fig. 18: Mass spectrum of hexadecanoic acid methyl ester

Hexadecanoic acid (palmitic acid) is a saturated fatty acid .It is widely spread in plants and humans. This acid is produced first

during the synthesis of fatty acids and is considered as precursor of long-chain fatty acids. Palmitic acid is a major lipid component of human breast milk. The acid finds applications in soaps and cosmetics industries. It is also used in food industry .

Methyl stearate(7.75%)

Mass spectrum of methyl stearate is shown in Fig. 19.The The signal at m/z 298 with R.T. 17.786 corresponds $M^{+}[C_{19}H_{38}O_{2}]^{+}$ .The peak at m/z267 is due to loss of a methoxyl

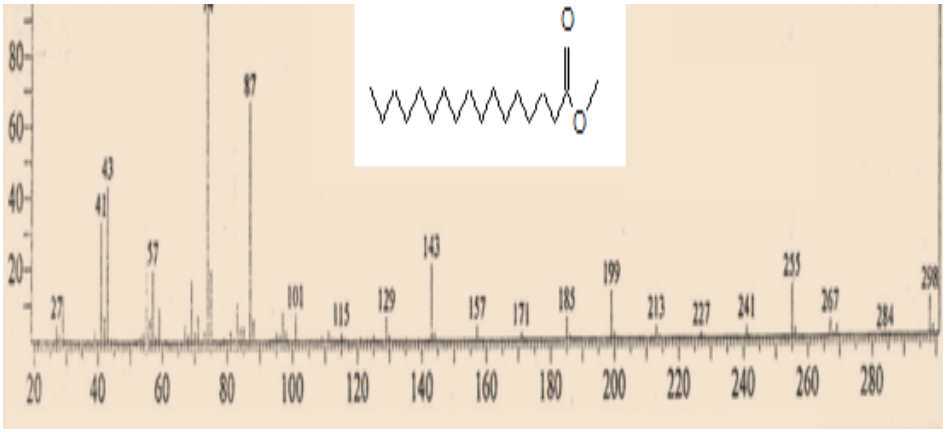


Fig. 19: Mass spectrum of methyl stearate

3.3.2-Antimicrobial activity

The mean diameters of inhibition zone (MDIZ) produced by oil on standard microorganisms are presented in Table (3.8). The results were interpreted in commonly used terms : < 9 mm considered inactive ; 9-12 mm partially active ; 13-18 mm active and more than 18 mm very active. Results displayed in Table 4 demonstrate activity of oil against all test organisms in the range : 100 - 50mg/ml. On the basis of its promising

antimicrobial activity, it seems that this oil is a lead for further opimization.

Table 3.8: Antimicrobial activity of *Cassia fistula* oil

Sample	Inhibition zone diameter (mm / mg oil)				
	Bs (G^+)	*Sa* (G^+)	*Ec.* (G^-)	*Pa* (G^-)	*Ca.*
Control Methanol	00	00	00	00	00
Cassia fistula oil (100mg/ml)	15	15	16	15	14
50mg/ml	13	14	13	14	13
25 mg/ml	13	13	9	13	12
12.500 mg/ml	12	12	--	10	--
6.25 mg/ml	10	7	--	8	--

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