

### 3-Results and Discussion

#### 3.1- Extraction of plant phenolics

Soxhlet extraction of *Albiza amara* roots gave 174 g of crude ethanolic extract which was successively partitioned with petroleum ether, chloroform, ethyl acetate, and n-butanol. Yields for these fractions are depicted in Table 3.1 (see also Fig.3.1).

Table 3.1: Yield of the different crude extracts of *A.Amara* root

Fraction	Extract in gram	Yield (%)
Petroleum ether	5	6.7
Chloroform	6.3	8.5
Ethyl acetate	9.7	12.8
n-Butanol	14.2	19.1

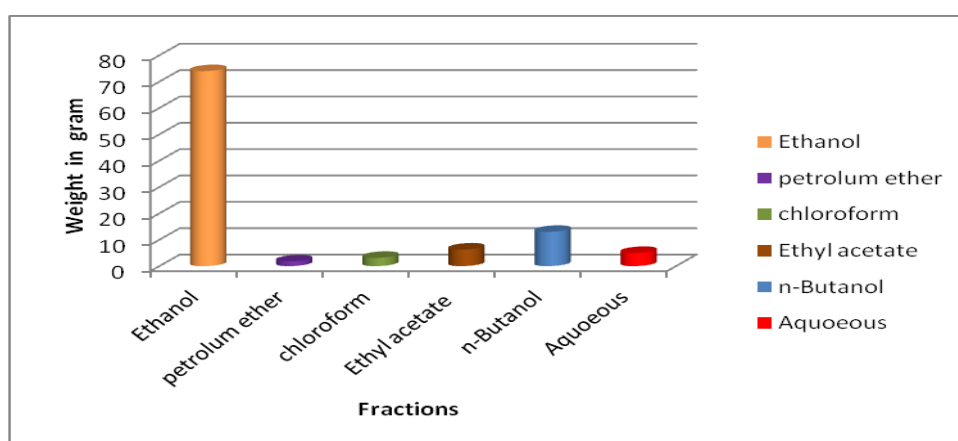


Fig.3.1: Weight of different fractions

### 3.2- Qualitative phytochemical analysis

The results of qualitative phytochemical analysis of chloroform, ethyl acetate, n-butanol and ethanolic extracts of *A.Amara* roots are given in Table 3.2. Results indicated the presence of many phyto-components in ethanolic and other fractions.

Table 3.2: Phytochemical screening of different fractions

No	Constituent	Test	Results				
			ET	PE	CH	EA	BU
1	Carbohydrates	Molish's	+++	-	-	-	+++
2	Flavanoids	Alkaline reagent	++++	-	++++	++++	-
3	Tannins,Phenolic	Ferric chloride Lead acetate	+++++	+++	++	+++	++
4	Saponins	Forth	+++	-	-	-	-
5	Alkaloids	Mayer's,Wagner's reagent	++++	-	++	++	++++

(+++)- Heavy; (++) - Medium ; (+) - Low ; (-) indicates absent

ET= ethyl acetate; PE= petroleum ether ; CH= chloroform; Bu= butanol

### 3.3-GC-MS analysis

Different extracts were subjected to GC-MS analysis. Volatile substances varied according to solvent. The ethyl acetate fraction yielded highest amount of volatile compounds (17compounds), petroleum ether (4 compounds), chloroform (2 compounds),ethanol(9compounds) and n-butanol(7compounds). 2, 2'-Methylene bis[6-(1,1dimethylethyl)- 4-ethyl-phenol was the most frequently occurring component being detected in chloroform, ethyl acetate , n-butanol and ethanolic extracts (26.56,21.04 and 7.73%).

Respectively).Also Hexadecanoic acid methyl ester was detected in ethylacetate,n-butanol and ethanolic extracts (12.58, 15.07, 22.82% respectively) . Thirty two compounds were identified in *A.Amara* roots extract by GC-MS analysis. The percentage of volatile components in different fractions is shown in Fig.3.2. The active principles in ethyl acetate fraction with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are shown in Table 3.3.The corresponding chromatograms are shown in Fig.3.3.

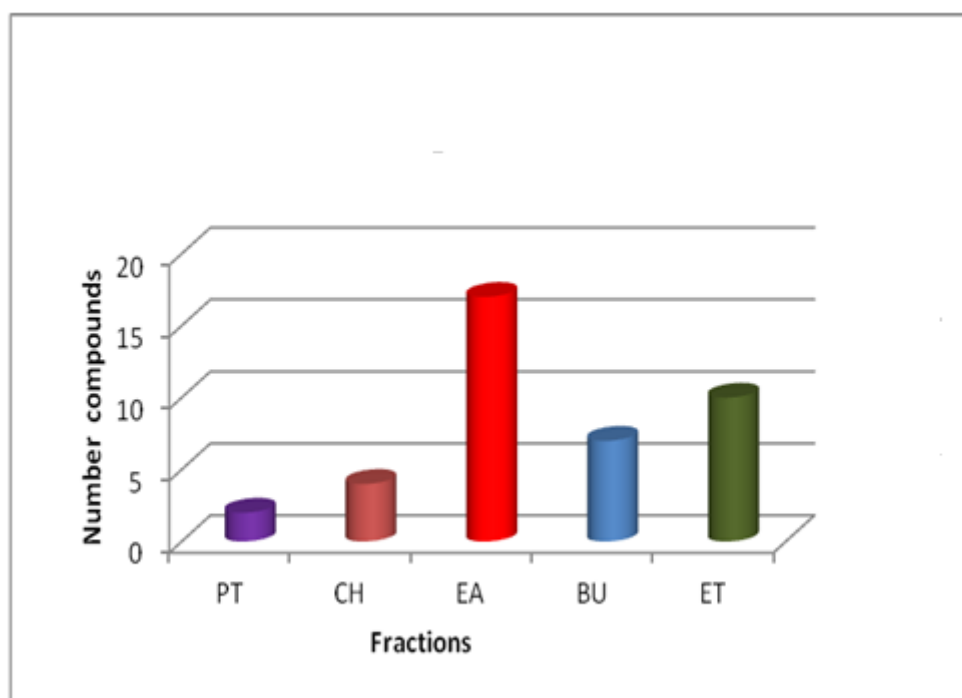


Fig.3.2: Volatile components in different fractions

Table 3.3: Volatile compounds in ethyl acetate fraction

Compound	Percentage of compound in fractions (%)					RT	MF
	PE	CH	EA	BU	ET		
Hexadecanoic acid, 14-methyl-, methyl ester	54.0	-	-	14.04	-	8.393	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>
Tetrasiloxane, decamethyl-	46.0	-	-	-	-	19.385	C <sub>10</sub> H <sub>30</sub> O <sub>3</sub> Si
Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-ethyl-	-	26.56	21.4	7.73	-	12.244	C <sub>25</sub> H <sub>36</sub> O <sub>2</sub>
1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	-	15.71	-	-	-	12.558	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>
1,2-Bis(trimethylsilyl)benzene	-	11.84	-	-	-	14.407	C <sub>6</sub> H <sub>4</sub> [Si(CH <sub>3</sub> ) <sub>3</sub> ] <sub>2</sub>
Trimethyl[4-(2-methyl-4-oxo-2-pentyl)phenoxy]silane	-	45.89	-	-	-	19.837	C <sub>15</sub> H <sub>29</sub> O <sub>2</sub> Si
Hexadecanoic acid, methyl ester	-	-	12.58	15.07	22	7.466	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>
Heptadecanoic acid, methyl ester	-	-	16.79	-	-	8.393	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>
9,15-Octadecadienoic acid, methyl ester, (Z,Z)-	-	-	3.09	-	-	8.959	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>
9-Octadecenoic acid, methyl ester, (E)-	-	-	12.83	16.06	-	9.039	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>
Heptadecanoic acid, 14-methyl-, methyl ester, (+/-)-	-	-	1.19	-	-	9.291	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>
Eicosane	-	-	2.05	-	-	10.069	C <sub>20</sub> H <sub>42</sub>
8-Methyl-6-nonenamide	-	-	2.46	-	-	11.088	C <sub>18</sub> H <sub>27</sub> NO <sub>3</sub>
1-Bromoeicosane	-	-	2.75	-	-	14.653	C <sub>20</sub> H <sub>41</sub> Br
Cyclotrisiloxane, hexamethyl-	-	-	1.3	0.19	-	15.339	C <sub>6</sub> H <sub>18</sub> O <sub>3</sub> Si
1,4-Phthalazinedione, 2,3-dihydro-6-nitro	-	-	4.5	-	-	19.110	C <sub>8</sub> H <sub>5</sub> N <sub>3</sub> O <sub>4</sub>
2-p-Nitrophenyl-oxadiazol-1,3,4-one-5	-	-	2.51	-	--	19.838	C <sub>8</sub> H <sub>5</sub> N <sub>3</sub> O <sub>4</sub>
9-Octadecenamide, (Z)-	-	-	-	42.12	-	11.071	C <sub>18</sub> H <sub>35</sub> NO
2,4,6-Cycloheptatrien-1-one, 3,5-bis(trimethylsilyl)-	-	-	-	4.79	-	15.048	C <sub>13</sub> H <sub>22</sub> OSi <sub>2</sub>
n-Hexadecanoic acid	-	-	-	-	1.5	7.758	C <sub>16</sub> H <sub>22</sub> O
Heptadecanoic acid, methyl ester	-	-	-	-	38.2	8.399	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>
Octadecanoic acid, methyl ester	-	-	-	-	2.5	9.297	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>
5-Methyl-2-trimethylsilyloxy-acetophenone	-	-	-	-	1.4	15.402	C <sub>15</sub> H <sub>18</sub> O <sub>2</sub> Si
Cyclodecasiloxane, eicosamethyl-	-	-	-	-	1.1	9.818	C <sub>20</sub> H <sub>60</sub> O <sub>10</sub> Si
1,3-Diphenyl-2-azafluorene	-	-	-	-	2.8	9.715	C <sub>24</sub> H <sub>17</sub> N
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	-	-	-	-	5.23	8.971	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>
Hexadecane	-	-	3.28	-	-	10.905	C <sub>17</sub> H <sub>34</sub>
Tetracosane	-	-	4.05	-	-	11.712	C <sub>24</sub> H <sub>50</sub>

Heptadecane	-	-	3.35	-	-	12.484	C <sub>17</sub> H <sub>36</sub>
Hexacosane	-	-	3.43	-	-	13.234	C <sub>26</sub> H <sub>54</sub>
Heptacosane	-	-	2.69	--	-	13.960	C <sub>27</sub> H <sub>54</sub>

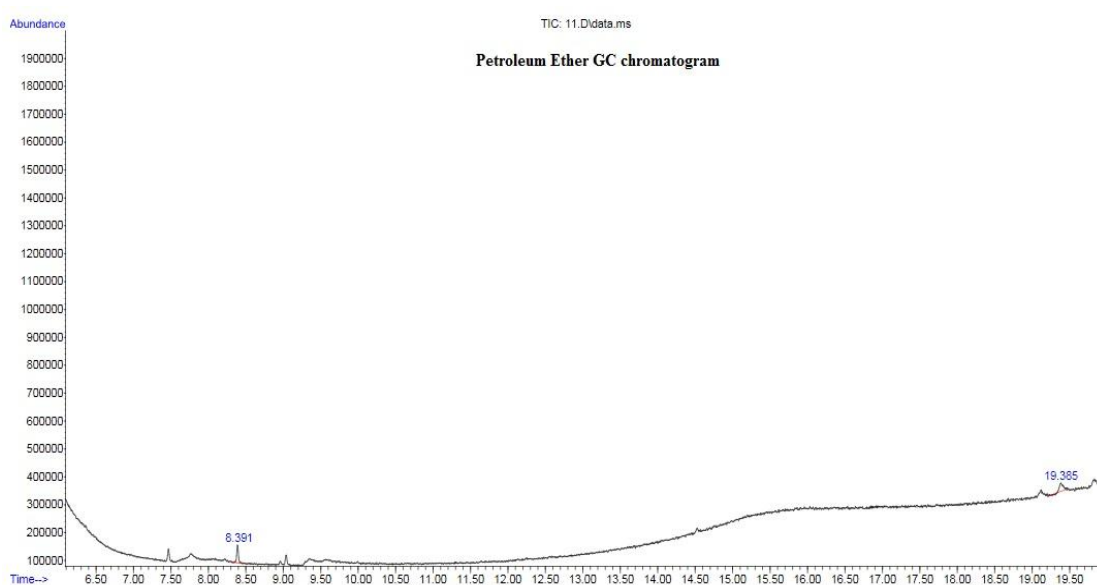


Fig.3.3: GC chromatograms for petroleum ether fraction

GC chromatograms for chloroform, ethyl acetate and n-butanol fractions are depicted in Figures 3.4-3.6.

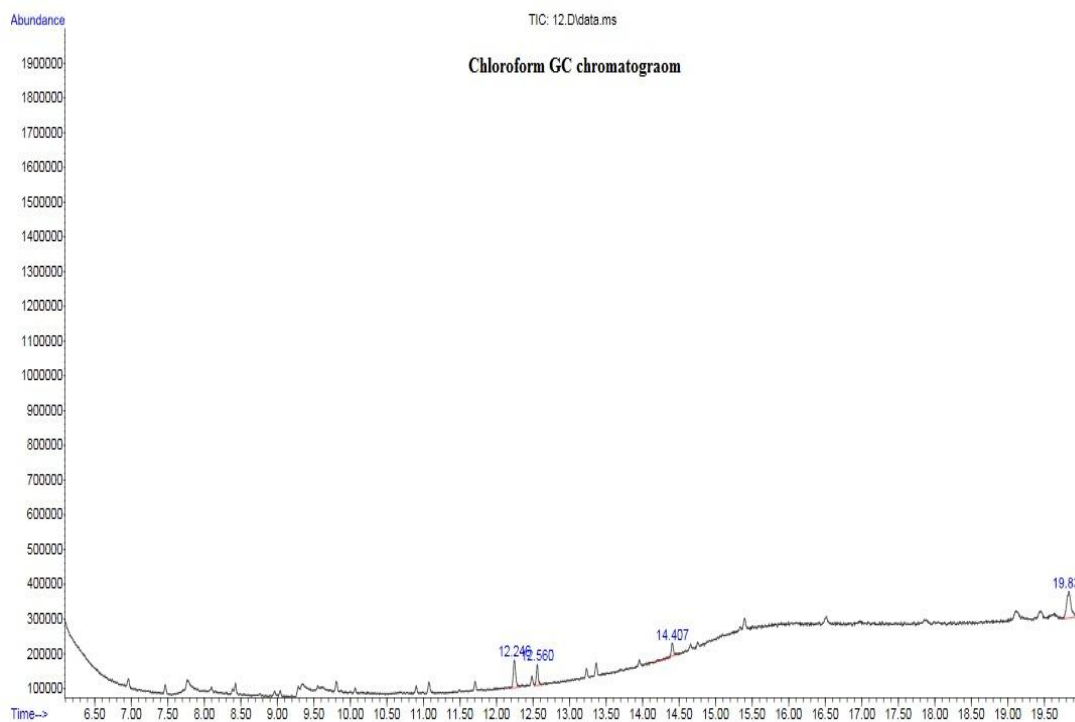


Fig.3.4: GC chromatograms for chloroform fraction

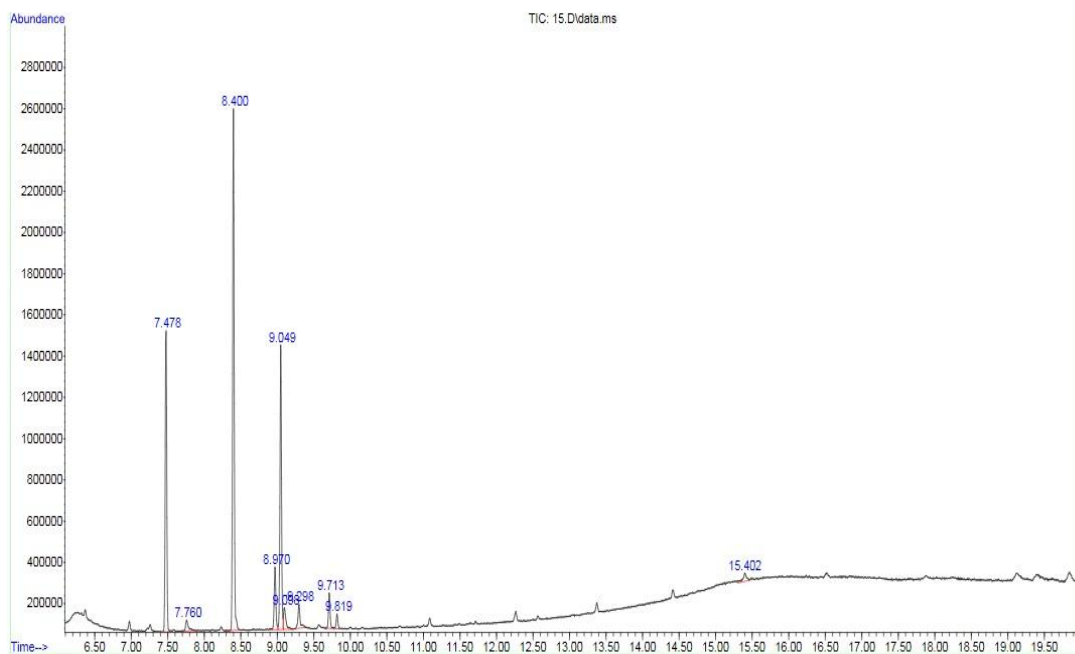


Fig.3.: GC chromatogram for ethyl acetate fraction

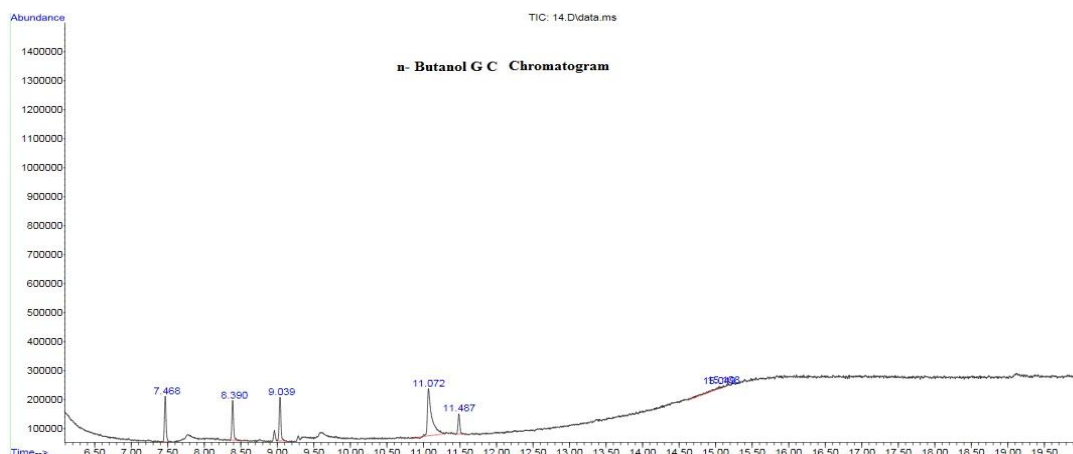


Fig.3.6: GC chromatograms for n-butanol fraction

### 3.4-Total antioxidant activity and total phenolic content of fractions:

The total antioxidant capacity (TAC) and total phenolic content (TPC) of different fractions are presented in table 3.4. The antioxidant activity of the *A.Amara* extracts were determined by different assays: DPPH, FRAP and CUPRAC using  $\mu\text{g}$  Trolox equivalents per g of sample ( $\mu\text{g TE/g dry weight DW}$ ). Total phenolic content were expressed as mg of Gallic acid equivalents per gram of dry weight (mg GAE/g dry weight DW). Table 3.4 shows the antioxidant activity of different fractions obtained by FRAB,TPC,DPPH, and ORAC methods. The results of DPP and ORAC assays ranged from (771.83 to 24.28 mg/g) and (1902.69 to 75.79 $\mu\text{g/g}$ ) respectively. The reducing power (FRAP) of ethanolic extract and its fractions ranged from 132.35 to 538.09  $\mu\text{g}$  of TE/g extract. The total phenolic content varied from 719.48 to

3825.47  $\mu\text{g/g}$  of dry weight of extract, expressed as Gallic. Overall, the results showed that the ethyl acetate fraction has highest antioxidant activity at a mean of (DPPH), 771.83  $\mu\text{g/g}$  ; (TPC), 3825.47  $\text{mg/g}$ ; (CUPRAC) 1902.686  $\mu\text{g/g}$ . (FRAP), 538.09  $\mu\text{g/g}$  . The chloroform fraction gave the lowest antioxidant activity at a mean of: (DPPH)  $\mu\text{g/g}$  24.28 ; (TPC) 719.48  $\mu\text{g/g}$  ; (CUPRA) 75.79  $\mu\text{g/g}$  ; (FRAP) 132.35  $\mu\text{g/g}$  (see Figures 3.7-3.11) .

Table 3.4: Total antioxidant capacity and total phenolic content of different extracts

$\mu\text{g g}^{-1}$	PT	CH	EA	BU	ET	$r^2$	equation
TPC	2085.29	719.48	3825.47	1738.29	2198.12	0.996	$y = 0.0039 x + 0.0577$
DPPH	311.59	24.28	771.83	227.16	339.27	0.998	$Y = 0.7344 x + 1.1155$
FRAP	424.97	132.35	538.09	343.82	440.79	0.999	$Y = 1.243 x + 0.1558$
CUPRAC	433.94	75.79	1902.68	663.28	624.01	0.9988	$y = 0.4244 x + 0.0525$

PT = petroleum ether , CH = chloroform , EA = ethyl acetate , BU = n- butanol , ET = ethanol

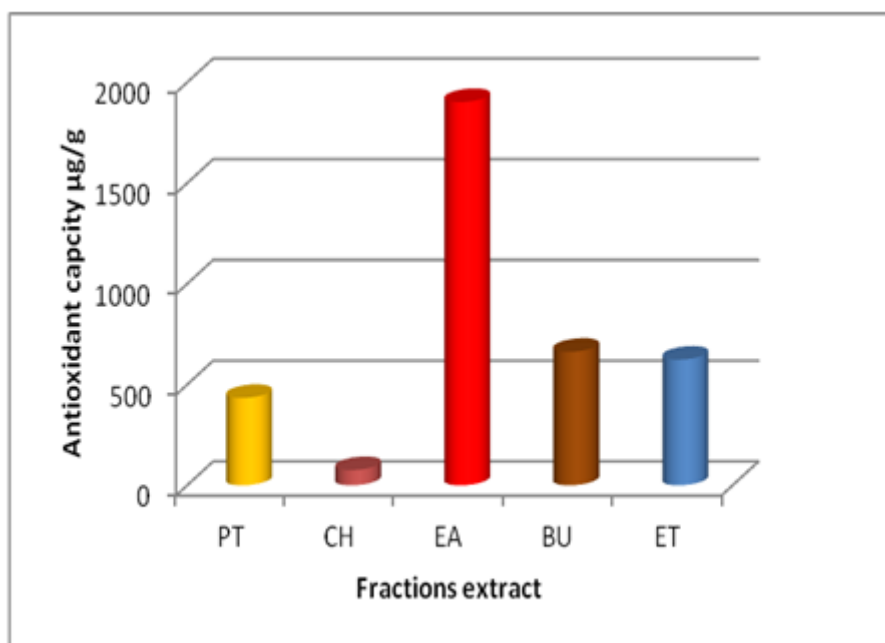


Fig.3.7: Antioxidant activity of different extracts using( CUPRAC) assay



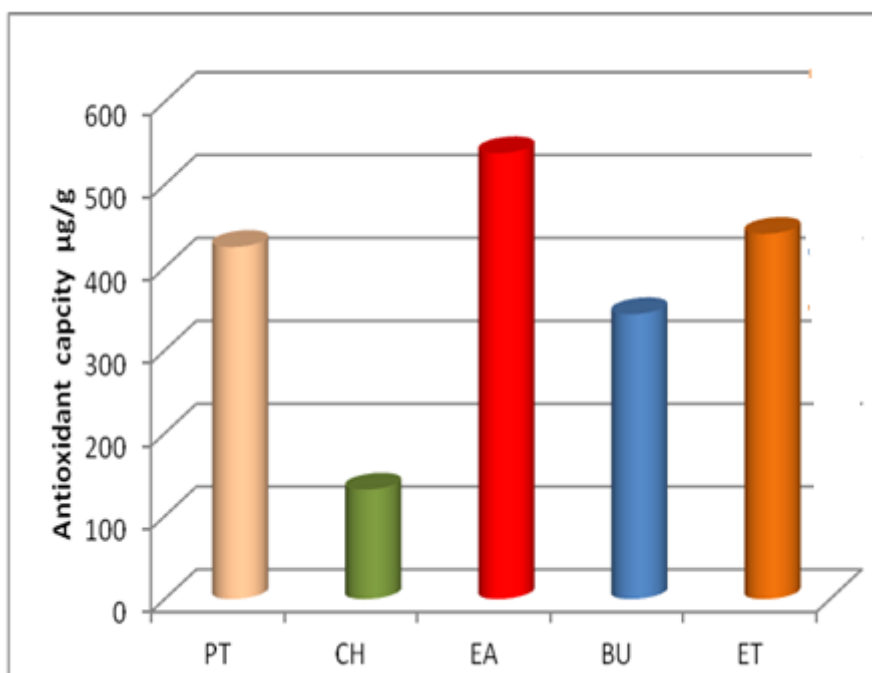


Fig.3.8: Antioxidant activity of different extracts using( FRAP) assay

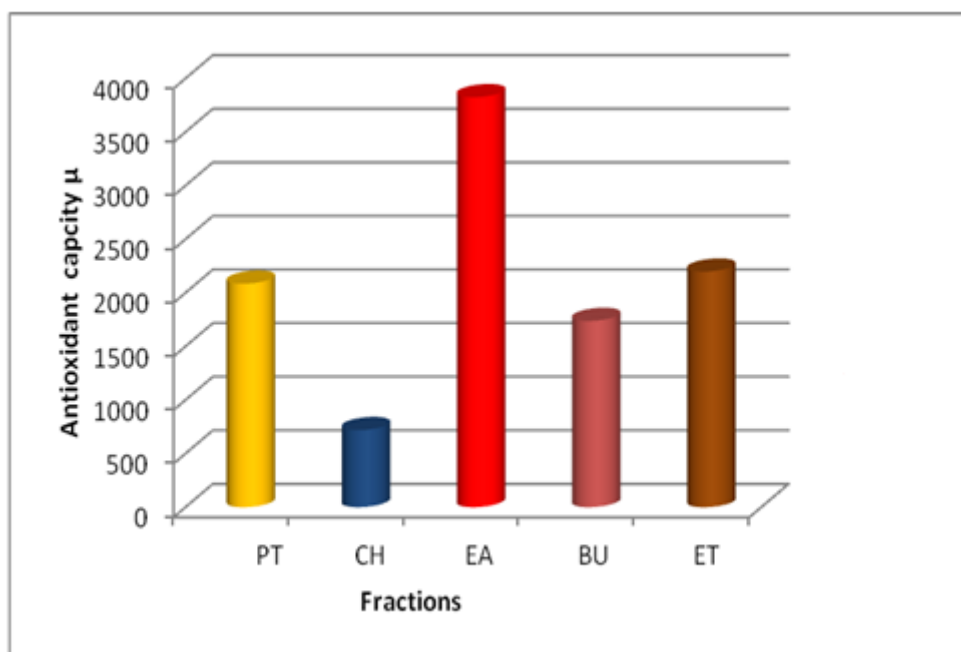


Fig.3.9: Antioxidant activity of different extracts using( TPC) assay

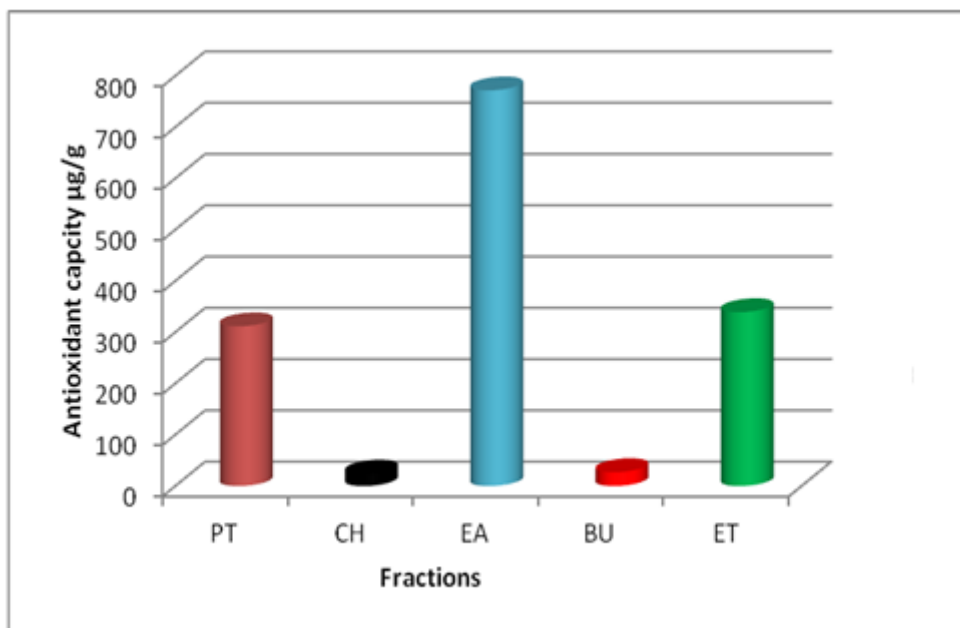


Fig.3.10: Antioxidant activity of different extracts using ( DPPH) assay

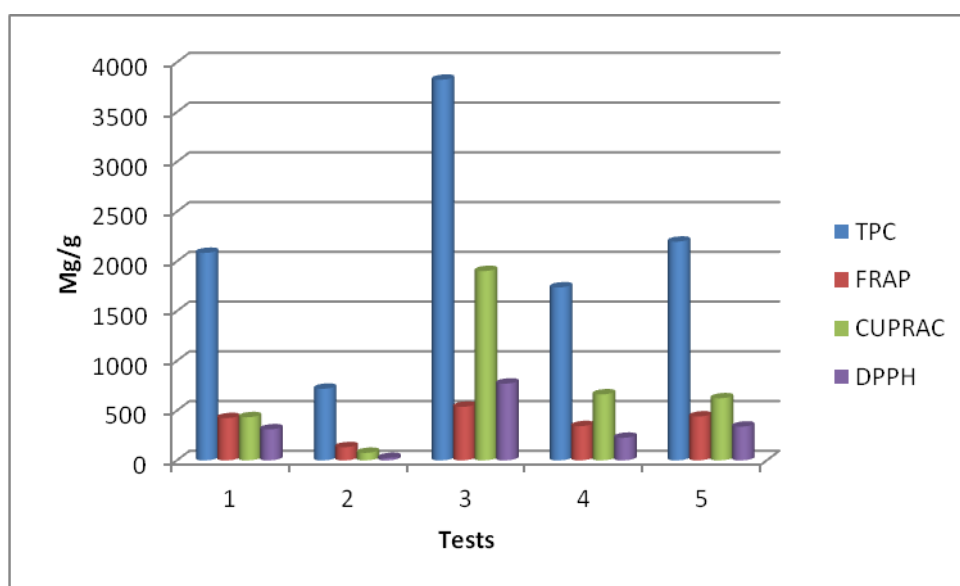


Fig.3.11: Total antioxidant activity determined by DPPH, TPC, FRAP and CUPRAC methods

### 3.5-Antibacterial activity of different fractions

In well diffusion method, all fractions from *Albizia. Amara* roots showed inhibitory activity against the *Streptococcus mutans* (SM) and *Lacto bacillus* (LB). The activity is expressed as less active, if the zone of inhibition is 9-12 mm, moderate: 15-16

mm and high if greater than 17 mm. The ethyl acetate extract showed high activity on *Streptococcus mutans* (SM), while the n-butanol extract showed high activity on *Lacto bacillus* (LB). The results of antimicrobial activity of the *A. amara* extracts against the microbial strains are depicted in Table 3.5.

DMSO also had no effect on the growth of any of the test microorganisms. Standard disc inhibited the growth of all the test microorganisms. There has been an increasing effect on microbial growth inhibition with increasing concentration of the extracts. However; the effects observed were less than those produced by the standard chemotherapeutics. Further research is in progress to isolate the active metabolites responsible for the activity in the extracts of *A.Amara* roots.

Table 3.5: Antibacterial activity of different fractions

Microrganism	Fractions	300µl	200 µl	100 µl
<i>Lacto bacillus</i> (LB)	PT	20	19	18
	CH	12	11	12
	EA	14	12	11
	BU	22	22	20
	ET	21	19	18
	AMP	25	25	25
	DMSO	-	-	-
<i>Streptococcus mutans</i> (SM)	PT	9	10	11
	CH	15	14	12
	EA	20	23	22
	BU	18	14	15
	ET	20	20	20
	AMP	25	25	25
	DMSO	-	-	-

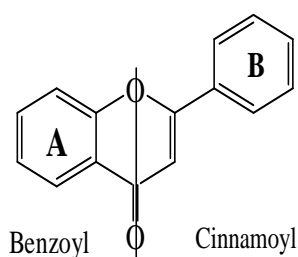
(ET = 0.09 , CH = 0.08, EA = 0.1 , P =0.03 , BU = 0.03) mg/ml , 100 µl of sample + ml of DMSO.

### 3.6-Identification of compound I

The barks of *Acacia mellifera* were macerated with 95% ethanol

at room temperature for 48hr. Removal of the solvent under reduced pressure gave a crude product. Paper chromatography of the crude extract gave a pure component – compound I.

Flavonoids usually exhibit two absorption bands in their UV spectra; band I and II. Band I is associated with the absorption of the cinnamoyl system, while band II originates from the benzoyl system. Flavones, flavonols, chalcones and aurones give band I and II, due to conjugation between the carbonyl function and the aromatic B ring. While flavanones, isoflavones, dihydroflavonols and dihydrochalcones give only band II in the range : 230-290nm. These classes of flavonoids lack conjugation between the B ring and the carbonyl function.



The UV absorption of flavones, flavonols, chalcones and aurones is depicted in Table 3.6.

Table 3.6: The UV absorption of some flavonoids<sup>1</sup>

Flavonoid class	Band I	Band II
Flavones	330-350	250-270

Flavonols	350-390	250-280
Chalcones	365-390	240-260
Aurones	390-430	240-270

In the UV compound I absorbs (Fig.3.12) at  $\lambda_{\max}$  275nm. Such absorption is characteristic of: isoflavones, flavanones, dihydrochalcones and dihydroflavonols. However, isoflavones are characterized by a shoulder in the range: 300-340nm and

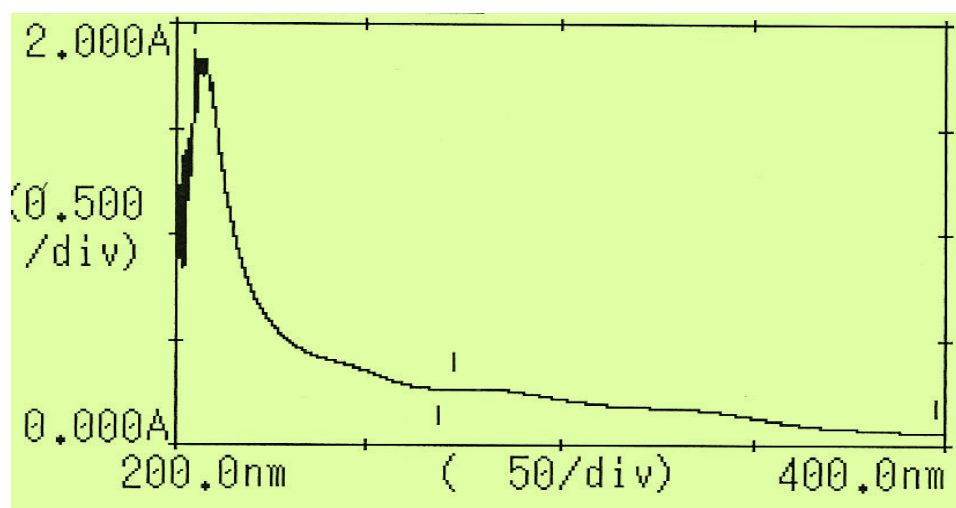


Fig.3.12: UV spectrum of compound I

such shoulder was not detected in the spectrum. Furthermore, the UV shift reagent-sodium methoxide- did not reveal any bathochromic shift in the spectrum(Fig.3.13). This indicates absence of a 3-OH group which is characteristic of dihydroflavonols. Sodium methoxide is a strong base and is used for the specific detection of 3- and 4`-OH functions.

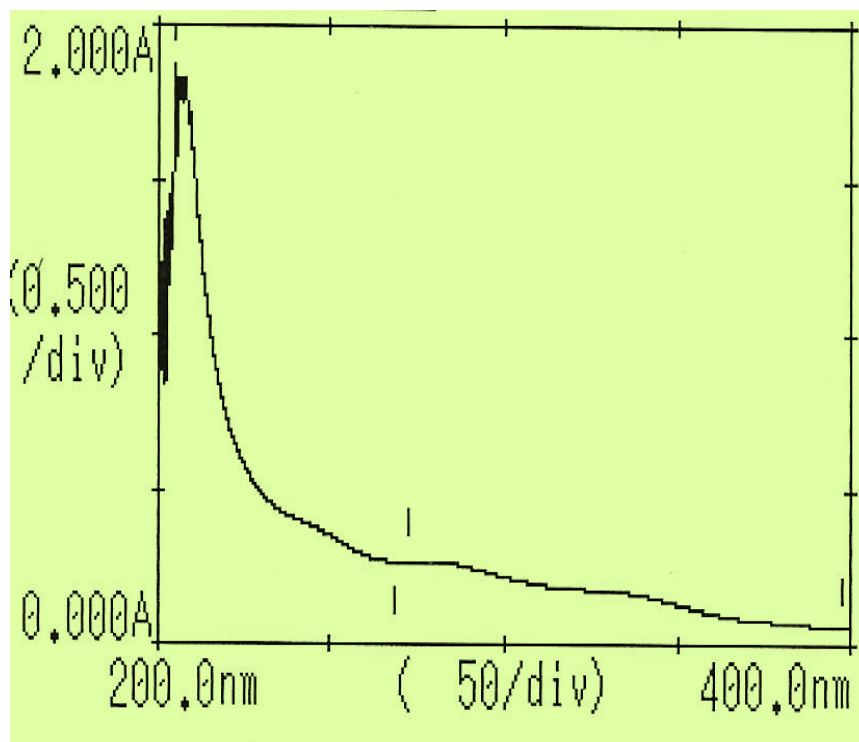


Fig.3.13: Sodium methoxide spectrum of compound I

The  $^1\text{H}$ NMR spectrum can differentiate between flavanones and dihydrochalcones. Due to the magnetically nonequivalent  $\text{C}_3$ -protons, which are split by  $\text{C}_2$  proton, a double multiplet usually appears in the spectra of flavanones at  $\delta 2.8$  and  $\delta 5.2$  ppm. Such multiplets were not observed in the  $^1\text{H}$  NMR spectrum (Fig.3.14) of compound I.

The sodium acetate spectrum did not reveal any bathochromic shift indicative of a 7-OH function (Fig.3.15).

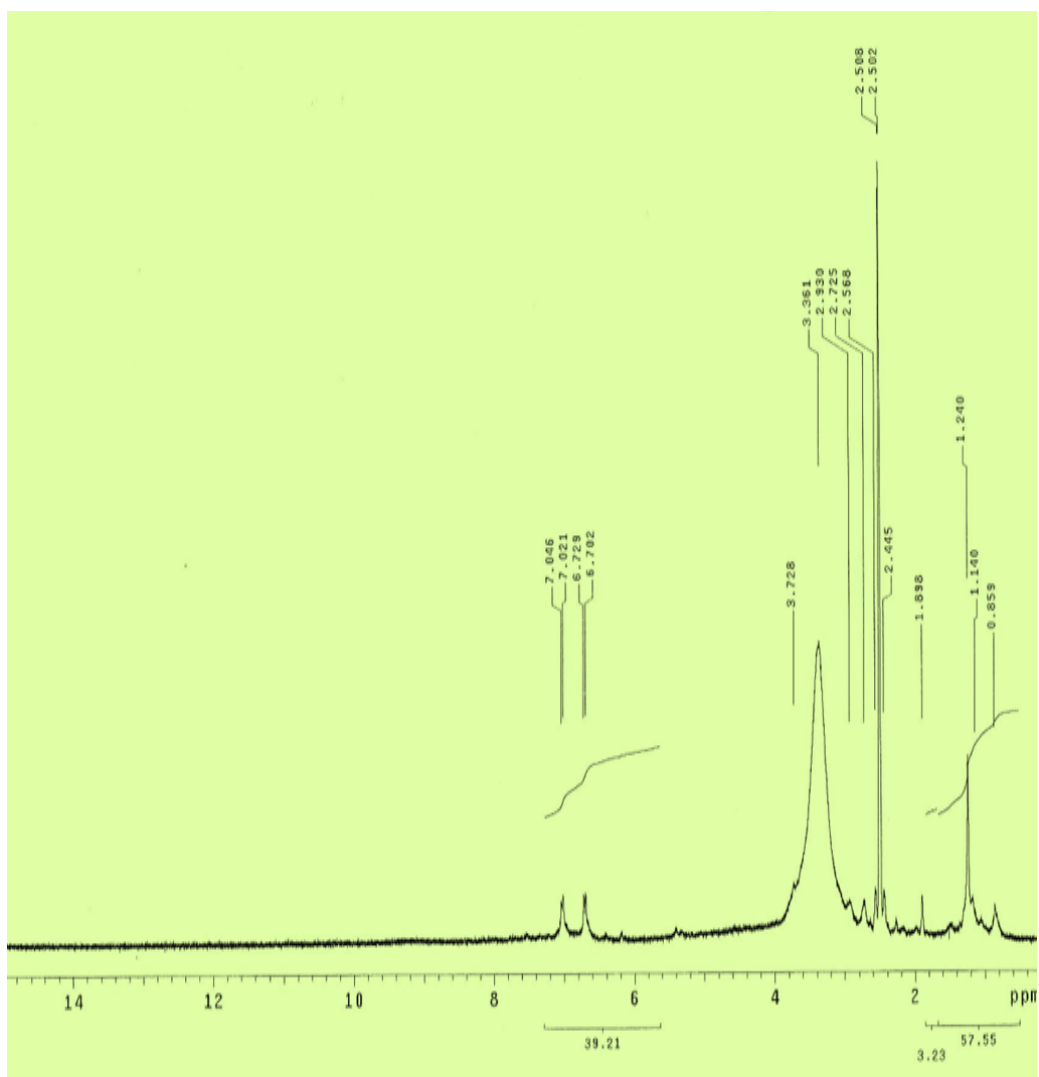


Fig.3.14:  $^1\text{H}$  NMR spectrum of compound I

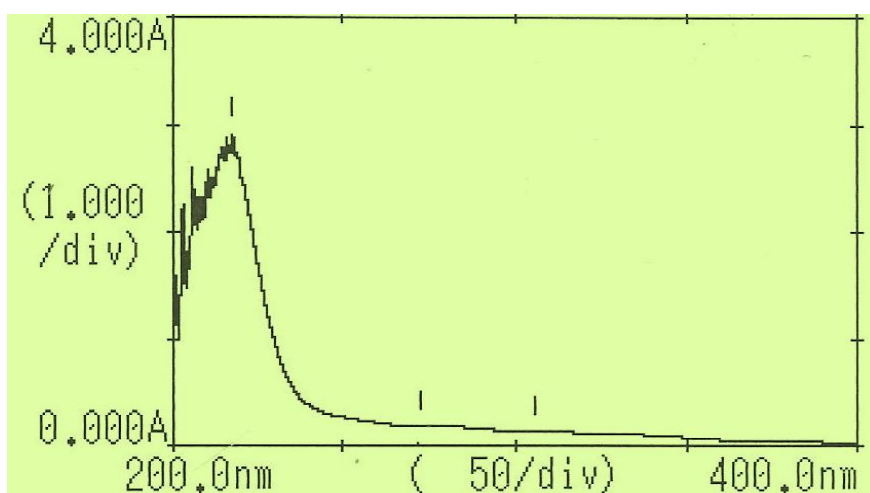


Fig.3.15: Sodium acetate spectrum of compound I

No bathochromic shift was detected in the aluminum chloride spectrum of this compound and this is indicative of absence of a 3- and 5-OH functions as well as catechol moieties in both aromatic rings (Fig.3.16).The same result was observed in the boric acid spectrum (Fig.3.17) which is diagnostic of catechol systems.

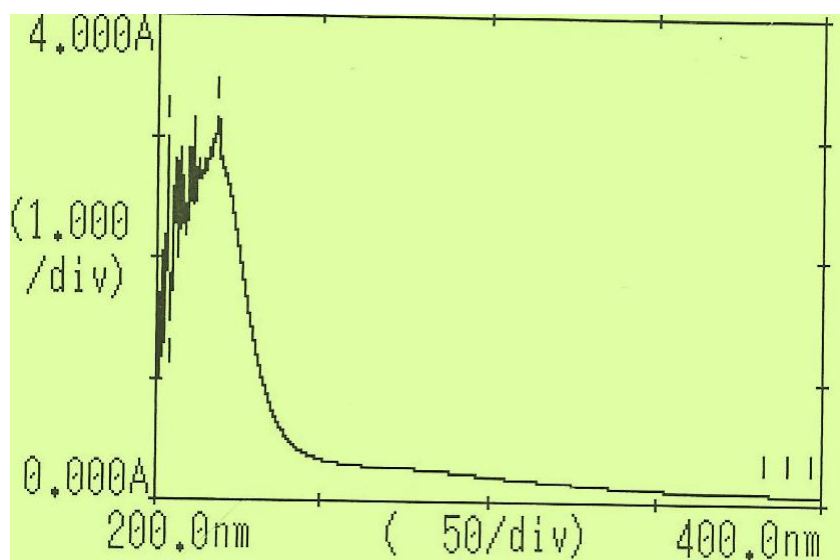


Fig.3.16: Aluminum chloride spectrum of compound I

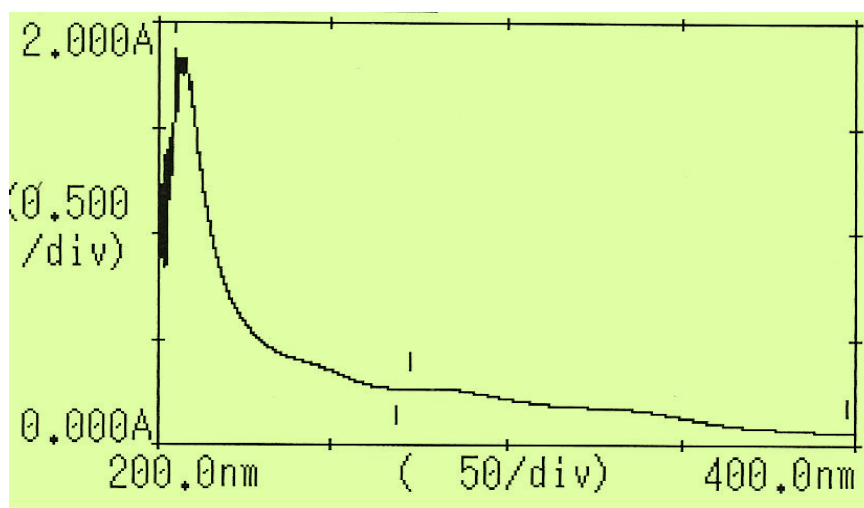
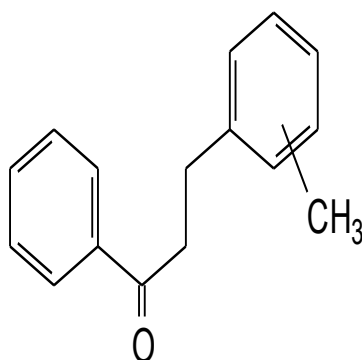


Fig.3.17: Boric acid spectrum of compound I



The above argument clearly indicates that this isolate is a dihydrochalcone. The  $^1\text{H}$ NMR spectrum (Fig.3.14) revealed a signal at  $\delta$ 0.85 (6H) assigned for a methyl group. The resonance at  $\delta$ 1.24 was assigned for the two methylene groups of the dihydrochalcone moiety. The A ring protons resonate at higher field relative to the B ring protons at  $\delta$ 6.70 due to the deshielding influence of the heterocyclic C ring on the neighbouring B aromatic ring. The signal at  $\delta$ 7.04 account for B ring protons.

On the basis of the above cumulative data the following tentative structure was proposed for this isolate:



Compound I

### 3.7-Identification of compound II

Compound II was isolated from the roots of *Albiza amara* as a pale yellow powder. The UV spectrum of compound II gave  $\lambda_{\text{max}}$ 281nm (Fig,3.18). This isolate is probably a dihydrochalcone

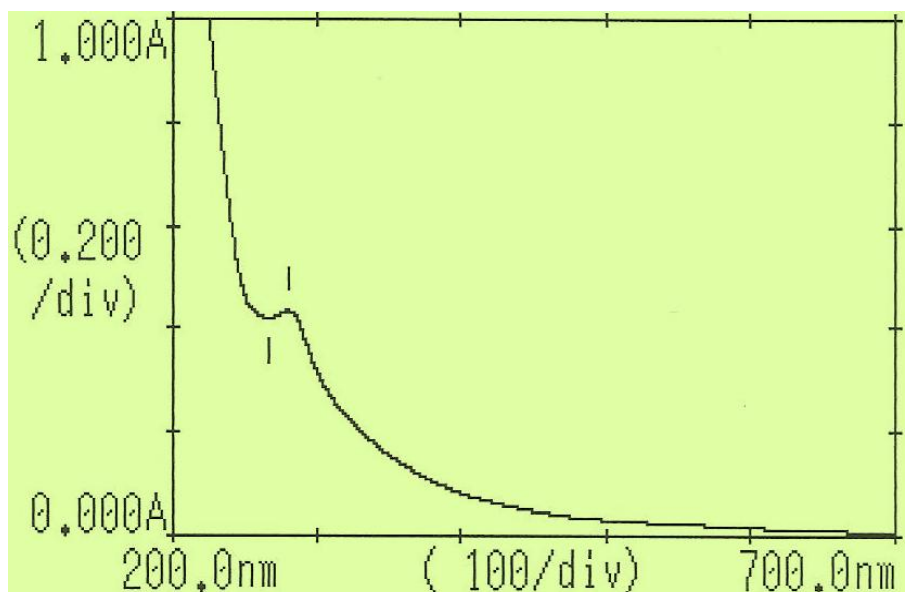


Fig.3.18:UV spectrum of compound II

since: (i) no shoulder characteristic of isoflavones was detected in the UV spectrum in the range 300-340nm;(ii) the 3-OH function was not detected in the sodium methoxide spectrum(Fig.3.19).This spectrum did not reveal any bathochromic shift.Furthermore the  $^1\text{H}$ NMR(Fig.3.20) did not reveal a pair of multiplets characteristic of flavanones around 2.8 and 5.2ppm.

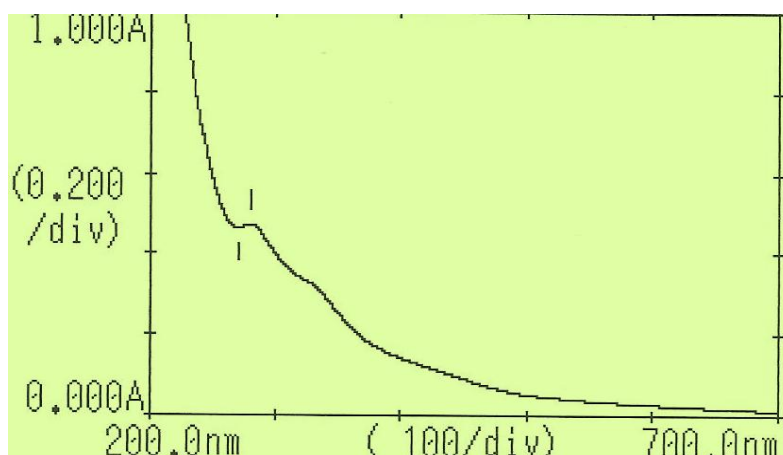


Fig.3.19: Sodium methoxide spectrum of compound II

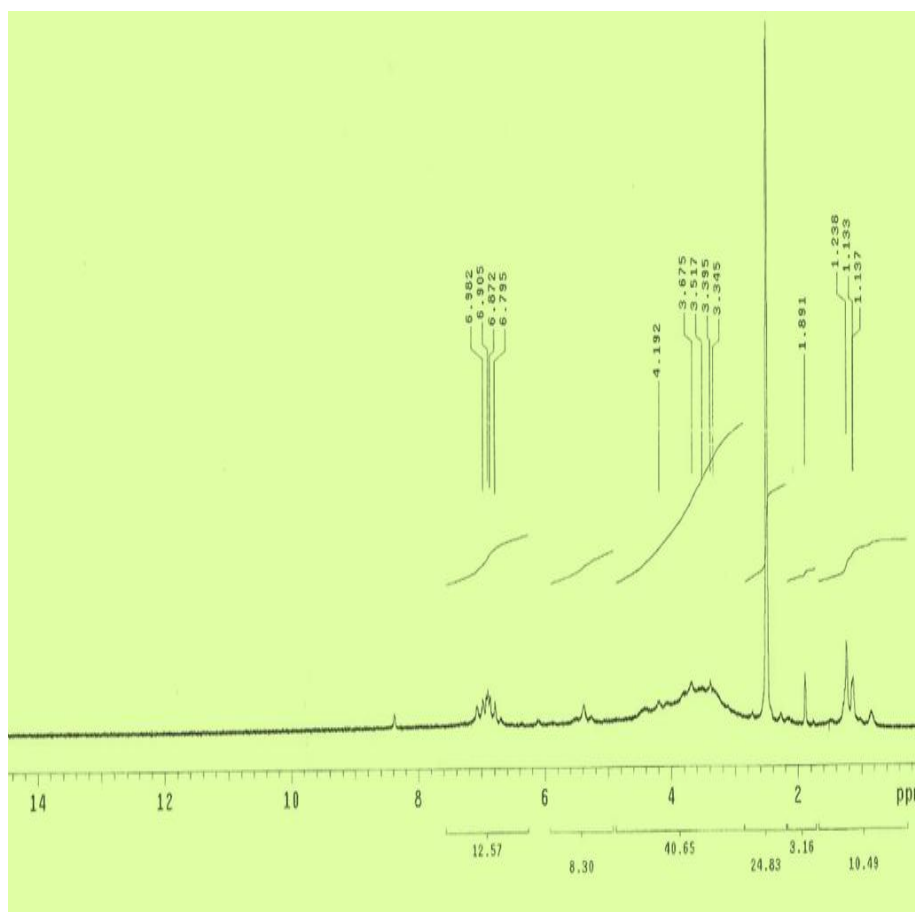


Fig.3.20:  $^1\text{H}$ NMR spectrum of compound I

Considerable structural features are gained by using the UV shift reagents: sodium acetate, aluminum chloride, hydrochloric acid and boric acid. No bathochromic shifts were observed in the sodium acetate spectrum (Fig.3.21); aluminium chloride spectrum (Fig.3.22) and boric acid spectrum (Fig.3.23). Such data indicate absence of hydroxylation at  $\text{C}_7$  (sodium acetate spectrum),  $\text{C}_{3,5}$  (aluminium chloride spectrum). The boric acid spectrum confirmed absence of catechol systems.

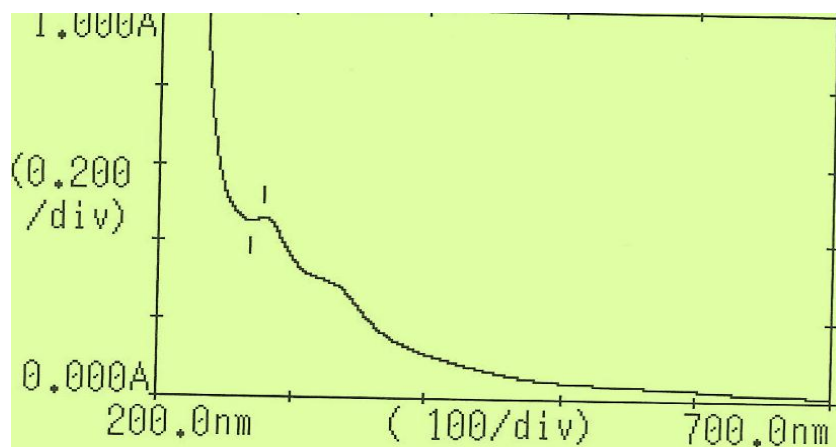


Fig.3.21: Sodium acetate spectrum of compound II

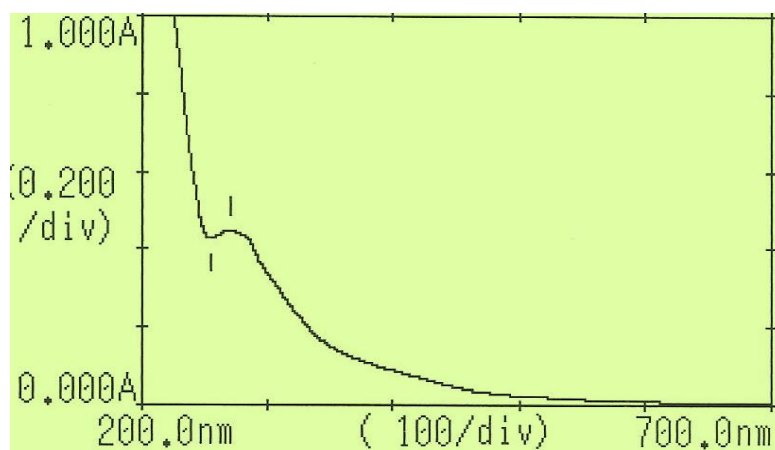


Fig.3.22: Aluminium chloride spectrum of compound II

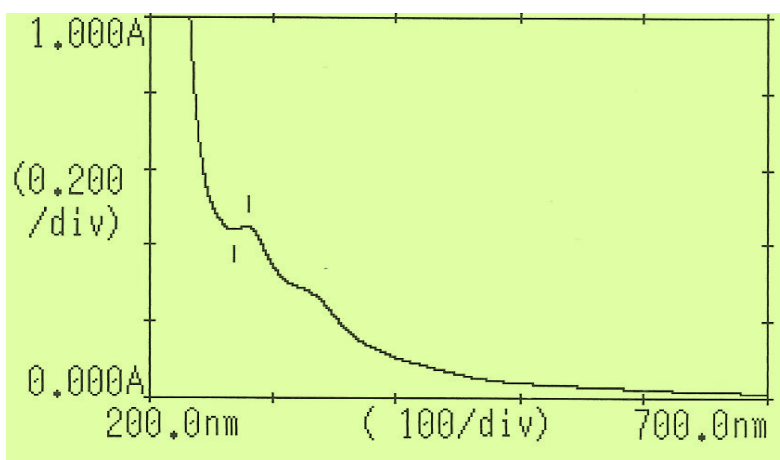
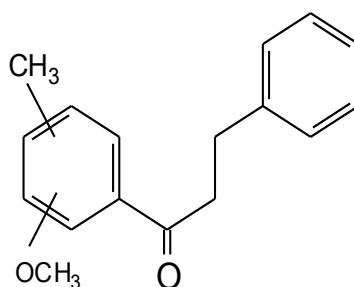


Fig.3.23: Boric acid spectrum of compound II

The  $^1\text{H}$ NMR spectrum (Fig.3.20) showed:  $\delta$ 1.13 (d,3H) accounting for a methyl group;  $\delta$ 3.67(s,3H) assigned for a methoxyl function;  $\delta$ 6.79(d,1H) due to  $\text{C}_6$  proton. The multiplet at 6.87-6.98 was assigned for the aromatic protons.

The mass spectrum (Fig.3.24) showed  $m/z$ 253 for  $\text{M}^+ - \text{H}^+$ . The retro Diels –Alder fission (Scheme I) revealed peaks at  $m/z$  166 and  $m/z$ 104 for intact A and B rings respectively. Such cleavage supports the following structure proposed for compound II:



Compound II

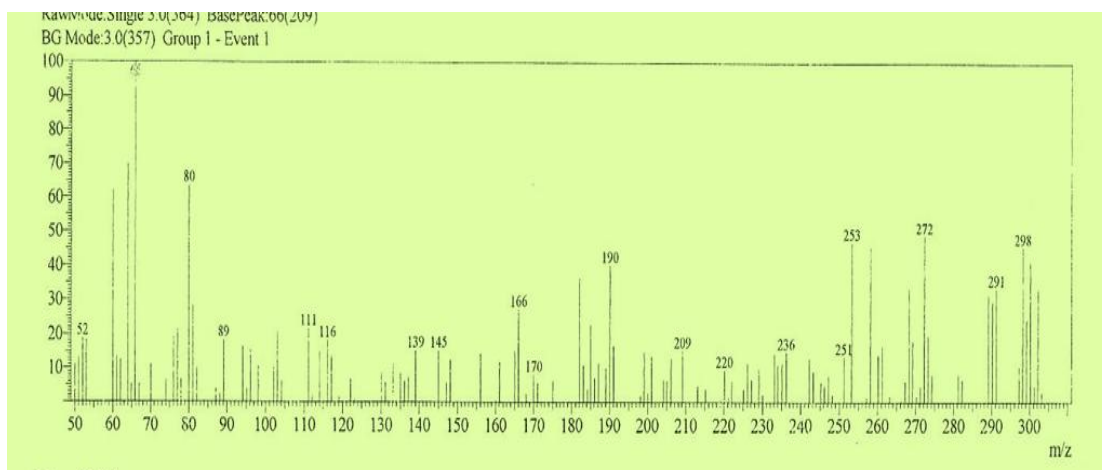
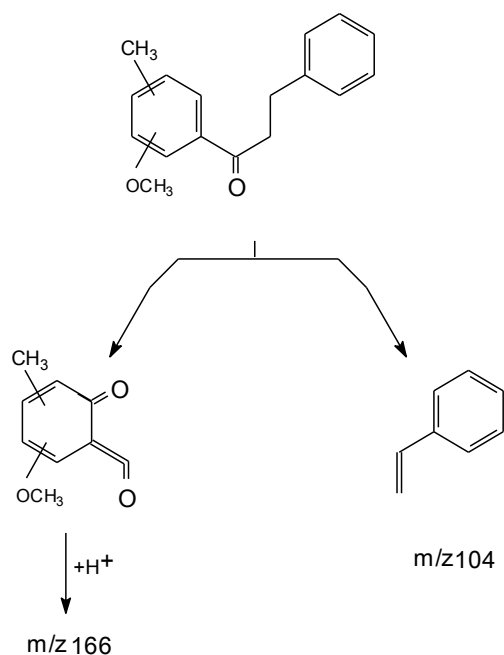


Fig.3.24: Mass spectrum of compound II



Scheme 3.1: Retro Diels-Alder fission of compound II

### 3.8-Identification of compound III

Compound III was obtained as yellow powder from the root extract of *Albiza amara*. The UV spectrum (Fig.3.25) of compound III showed  $\lambda_{\max}$  (MeOH) 256, 283 nm. Such absorption is shown by :flavanones, isoflavones, dihydroflavonol and dihydrochalcones.

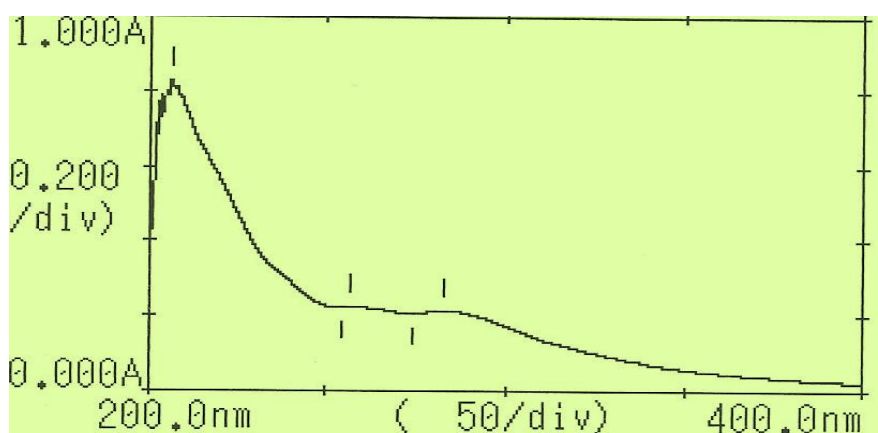


Fig.3.25: UV spectrum of compound III

The 300-340nm shoulder of isoflavones was not detected in the UV spectrum(Fig.3.25). Also no bathochromic shift characteristic of dihydroflavonols was shown by the sodium methoxide spectrum(Fig.3.26). Furthermore the  $^1\text{H}$ NMR(Fig.3.27) did not reveal a pair of multiplets characteristic of flavanones around 2.8 and 5.2ppm. Hence this isolate is a dihydrochalcone.

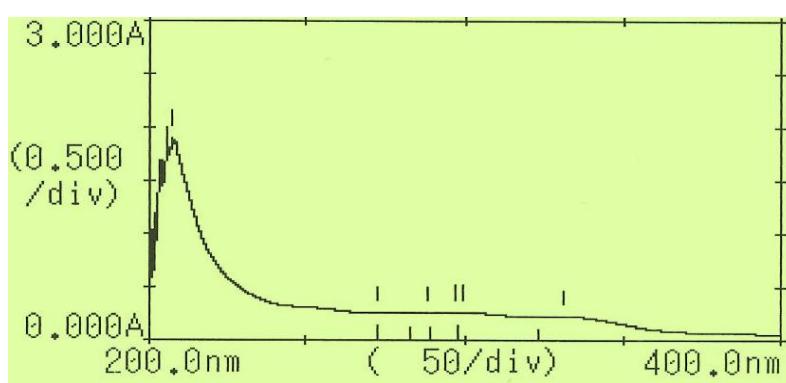


Fig.3.26 : Sodium methoxide spectrum of compound III

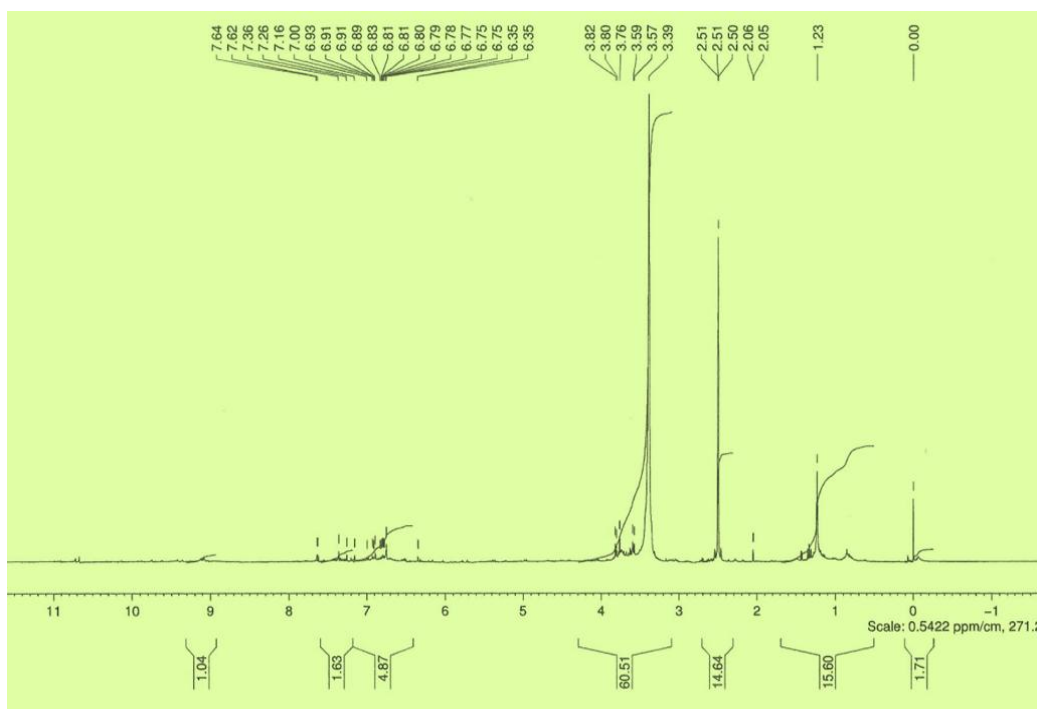


Fig.3.27:  $^1\text{H}$ NMR spectrum of compound III

The  $^1\text{H}$ NMR spectrum revealed a three proton signal at  $\delta$ 1.23ppm assigned for a methyl group .The resonances at  $\delta$ 3.76 (3H) and 3.80(3H)ppm account for two methoxyls .The  $\text{C}_6$  and  $\text{C}_8$  protons resonate at  $\delta$ 6.30 and  $\delta$ 6.80ppm, while the  $\text{C}_5$  proton appears as singlet at  $\delta$ 6.90ppm.The resonances at  $\delta$ 7.00(1H),7.16(1H) and  $\delta$ 7.60ppm(1H) are characteristic of aromatic protons.

When sodium acetate was added to a methanolic solution of compound III no bathochromic shift was observed (Fig.3.28) indicating absence of 7-OH function.

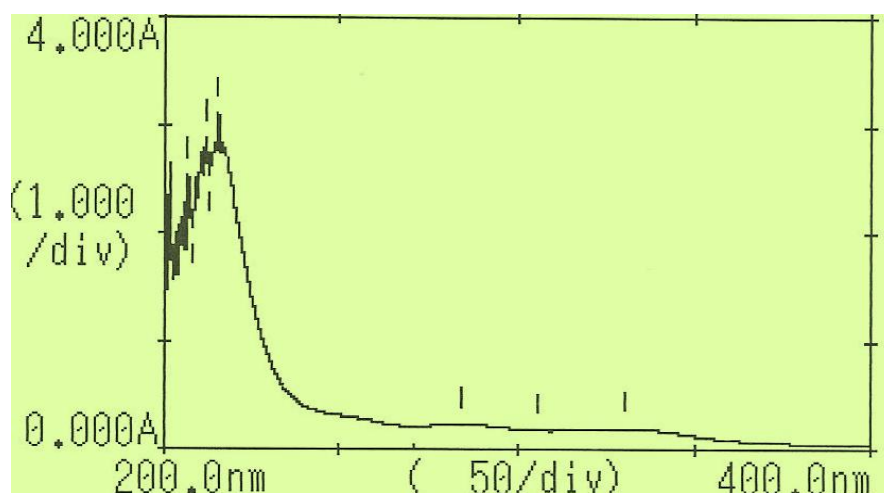


Fig.3.28: Sodium acetate spectrum of compound III

Addition of aluminium chloride to a methanolic solution of compound III revealed no bathochromic shift indicative of a 3- and 5-OH functions (Fig.3.29) .



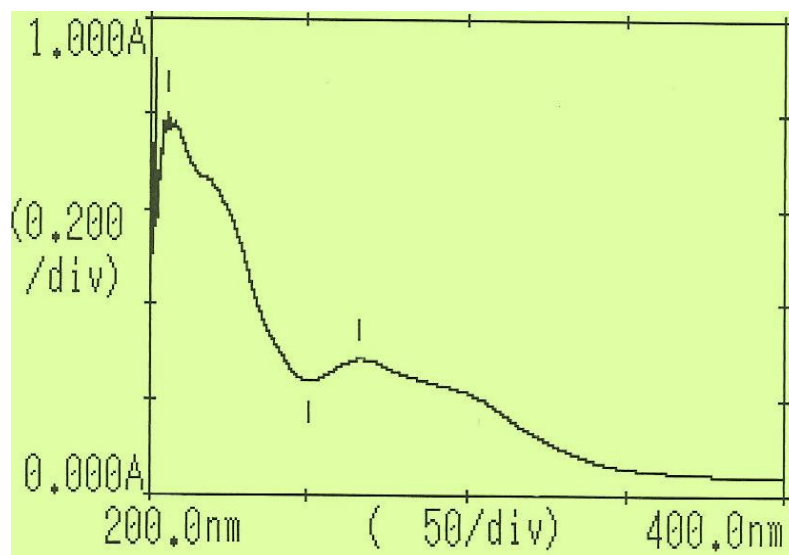


Fig.3.29 : Aluminium chloride spectrum of compound III

Also no bathochromic shifts were observed in the boric acid spectrum (Fig.3.30) which is diagnostic of catechol moieties.

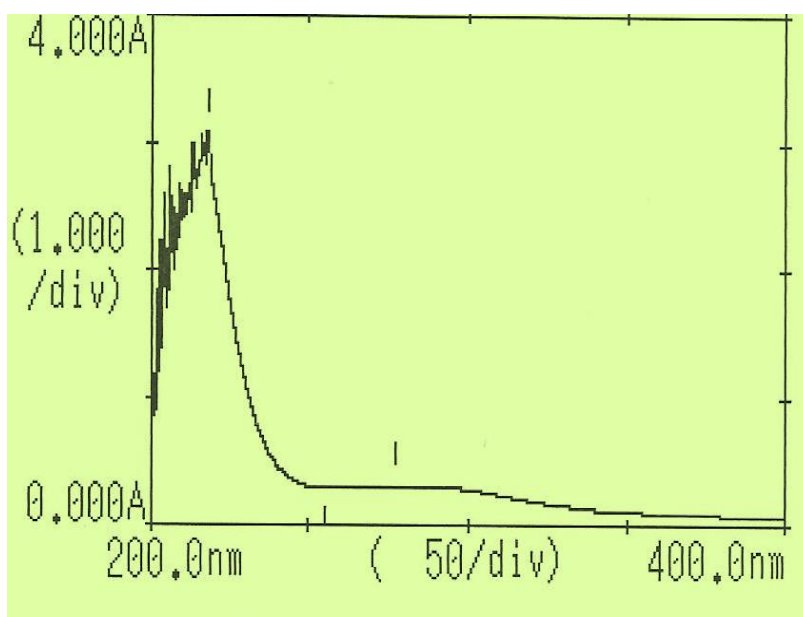
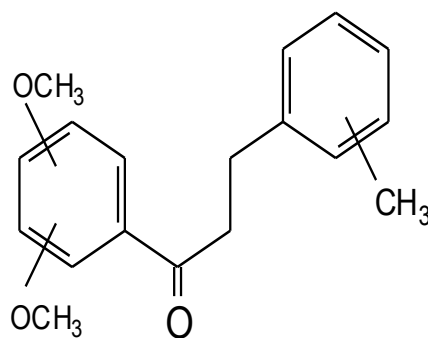


Fig.3.30: Boric acid spectrum of compound III

On the basis of such cumulative data the following tentative structure was proposed for this dihydrochalcone:



Compound III

### 3.9-Identification of compound IV

Compound IV was isolated from the roots of *Albiza amara* by paper chromatography. The UV spectrum (Fig.3.31) of compound IV showed  $\lambda_{\max}$  275nm. Such absorption is characteristic of: flavanones, isoflavones, dihydroflavonols and

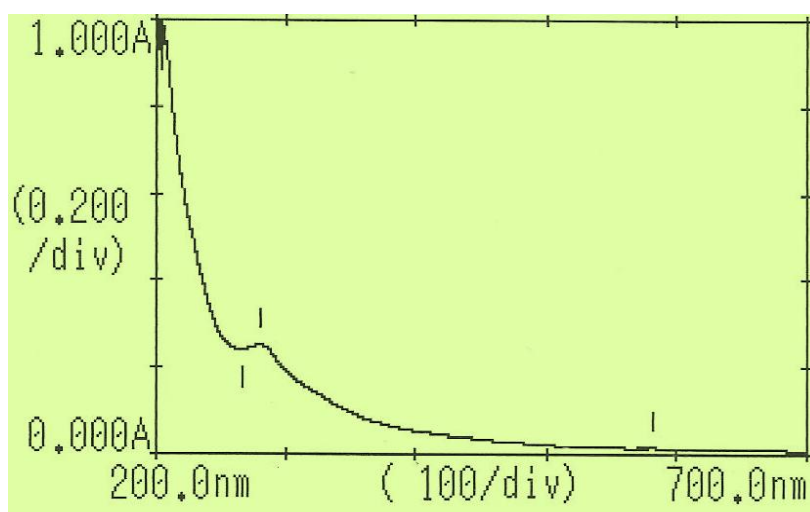


Fig.3.31 : UV spectrum of compound IV

dihydrochalcones. No bathochromic shift was observed when sodium methoxide was added to a methanolic solution of compound IV (Fig.3.32). This indicates absence of a 3-OH group

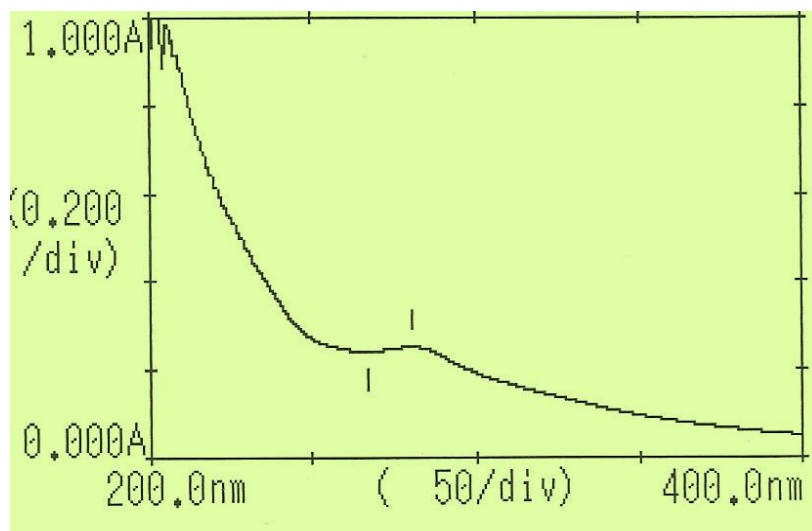


Fig.3.32: Sodium methoxide spectrum of compound IV

Which is characteristic of dihydroflavonols? Also that shoulder which characterizes isoflavones (in the range: 300-340nm) was not detected in the UV spectrum of IV. Furthermore the  $^1\text{H}$ NMR (Fig.3.33) did not reveal a pair of multiplets characteristic of flavanones around 2.8 and 5.2ppm. Hence this isolate is a dihydrochalcone.

The  $^1\text{H}$ NMR spectrum revealed a three proton signal at  $\delta$ 1.23ppm assigned for a methyl group. The resonance at  $\delta$ 3.76(3H) account for a methoxyl function. The resonates at  $\delta$ 6.70(3H) and  $\delta$ 6.80(2H)ppm was attributed to A ring protons, while the multiplet at  $\delta$ 6.89-6.95ppm is characteristic of B ring protons.

No bathochromic shift was observed in the sodium acetate spectrum. This suggests absence of a 7-OH function (Fig.3.34).

The aluminum chloride spectrum (Fig.3.35) did not show a bathochromic shift indicating absence of 3- and 5-OH functions as well as catechol systems. The same trend was observed in the boric acid spectrum (Fig.3.36) which is diagnostic of catechol systems.

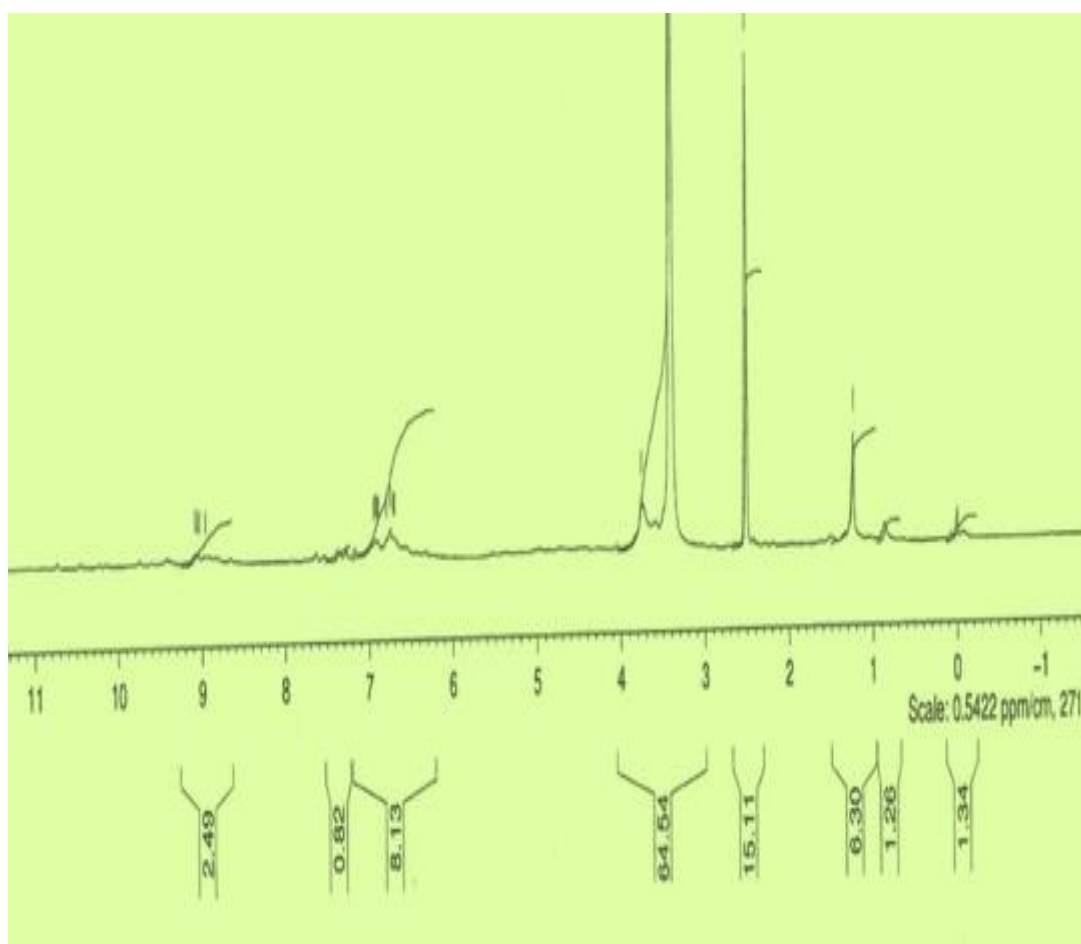


Fig3.33: <sup>1</sup>H NMR spectrum of compound IV

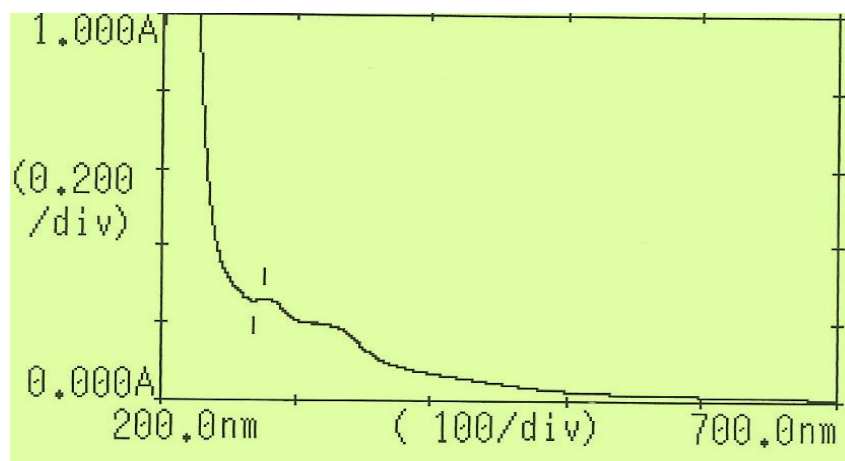


Fig.3.34: Sodium acetate spectrum of compound IV

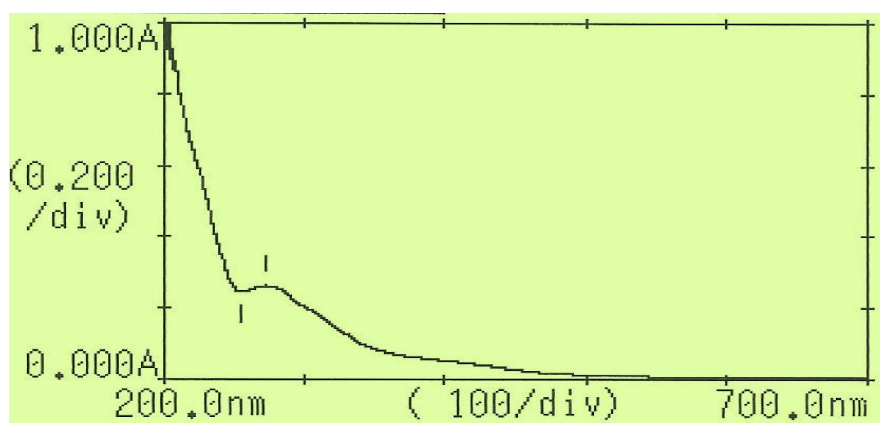


Fig.3.35: Aluminum chloride spectrum of compound IV

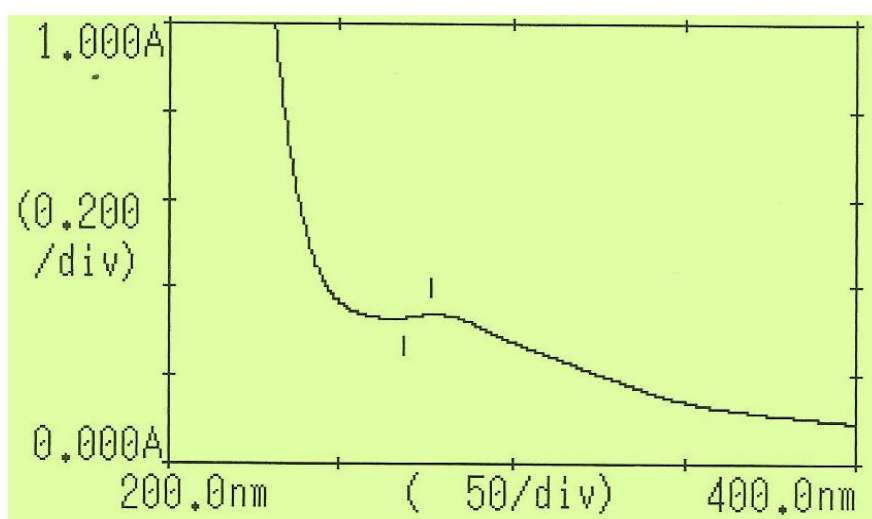
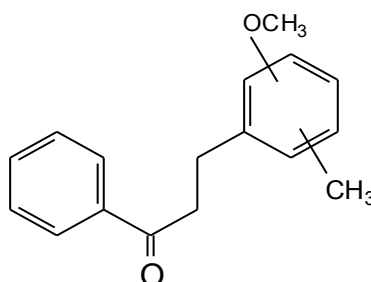


Fig.3.36: Boric acid spectrum of compound IV

On the basis of the above argument, the following tentative structure was suggested for compound IV:



Compound IV

### 3.10-Antibacterial activity

The isolated compounds were evaluated for their antibacterial and antioxidant potential. Two Gram negative bacterial strains- *Streptococcus mutans* (Sm) and *Lacto bacillus* (Lb)-

Were used in the Well diffusion method. The inhibitions zones are depicted in Table (3.7). Compound I showed significant activity against *Lacto bacillus* but it showed less activity towards *Streptococcus mutans* (Table 3.7-Fig.3.37). Compound II showed the same trend. However, compound IV gave significant activity against *Streptococcus mutans* and less activity towards *Lacto bacillus*. Compound III showed significant activity against all test organisms.

Table 3.7: Antibacterial activity of test compounds

Microorganism	Inhibition zone (mm)					
	I	II	III	IV	Standard amoxicillin	Control DMSO
SM	9	15	20	20	30	-
	10	14	23	20	30	-
	11	12	22	20	30	-
LB	17	20	25	15	25	-
	18	21	25	15	25	-
	18	33	23	14	25	-

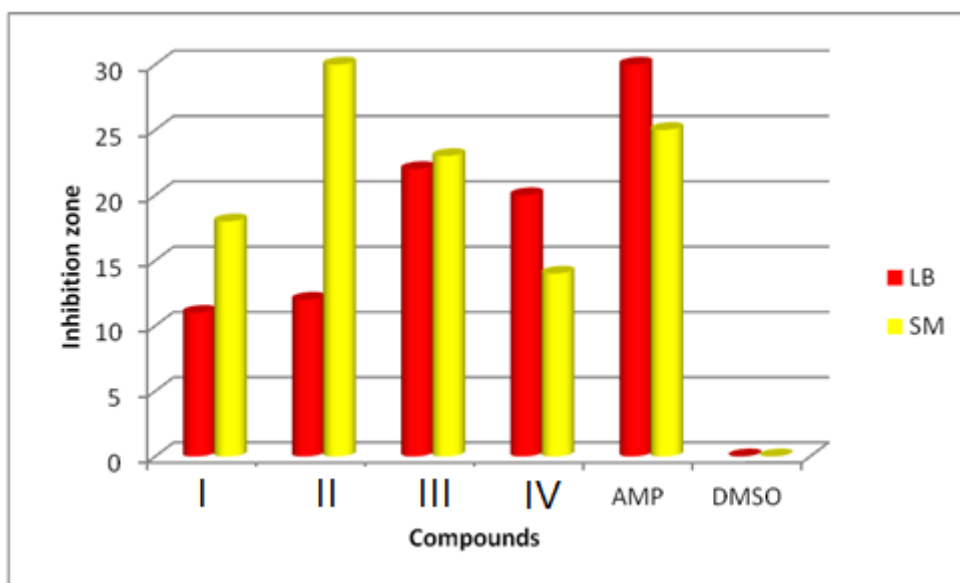


Fig.3.37: Antibacterial activity of compounds.

### 3.11-Anti-oxidant capacity

The antioxidant potential of the test compounds was assayed by two standard methods (DPPH and FRAP). For DPPH the percentage inhibition ranged from 80.47 to 89.71%. In the FRAP the inhibition concentration ranged from 72 to 218  $\mu\text{M/g}$  DW (see Table 3.8).

According to DPPH assay compound I showed the highest antioxidant potential (89.71%) followed by compounds II (86.76%) and III (85.10%). However, compound IV gave the least antioxidant activity (80.47%) (see Table 3.8 and Fig. 3.38).

In FRAP assay compound I gave the highest activity (inhibition conc. 72  $\mu\text{M/g}$  DW) followed by compounds II (110  $\mu\text{M/g}$  DW) and III (140  $\mu\text{M/g}$  DW). However, compound IV gave the least antioxidant activity (218  $\mu\text{M/g}$  DW) - see Table 3.8 and Fig. 3.39.

Table 3.8: Antioxidant activity of isolated compound (I-IV)

Compound	DPPH (%)Inhibition	(FRAP)Antioxidant con. in $\mu\text{M/g DW}$
I	89.71 $\pm$ .047*	72 $\pm$ .289*
II	86.76 $\pm$ 0.38*	110 $\pm$ .424*
III	85.15 $\pm$ 0.39*	140 $\pm$ .4210*
IV	80.47 $\pm$ 0.39*	218 $\pm$ .212*

\* Value represents mean  $\pm$  standard deviation ( $n=3$ ), which are significantly different at  $p<0.0$

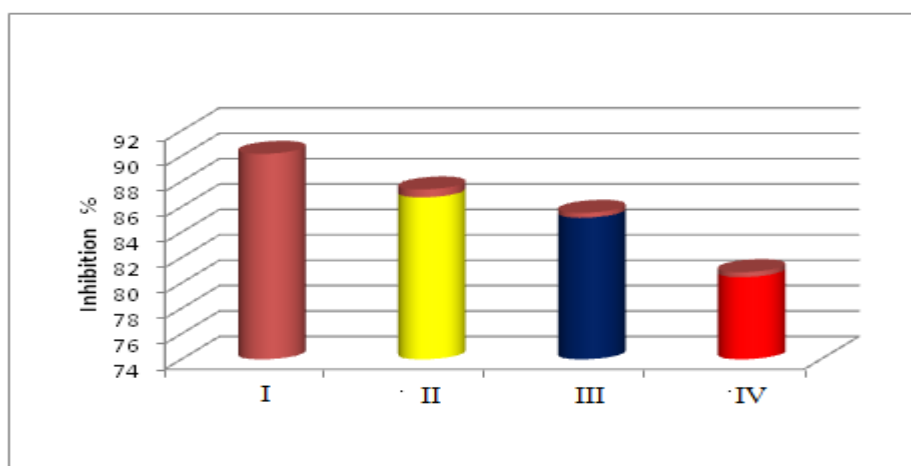


Fig.3.38: Percentage inhibition of test compounds (DPPH)

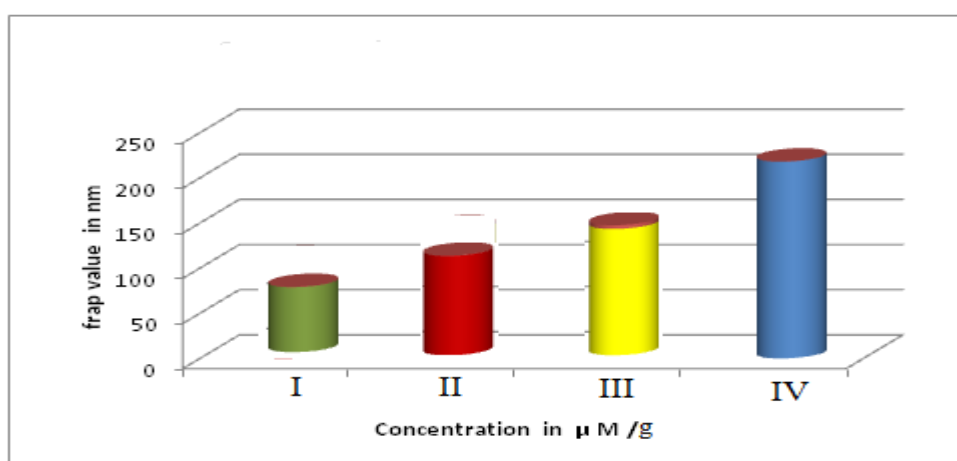


Fig. 3.39: Inhibition concentration of compounds using( FRAP) assay



## Conclusion

- This study indicated that the roots of *Albizia.Amara*, especially the ethyl acetate fraction is rich in antioxidants due to presence of phenolics .It seems that the plant is a good source of flavonoids as evidenced by the phytochemical screening.
- Phytochemical investigation of the ethanolic extracts and all fractions of *Albizia amara* Roots revealed the presence of important phytochemical in ethanolic and all fractions these are: alkaloids, terpenes, saponin , tannin and flavonoids.
- GC-MS analysis of *Albizia.Amara* roots showed ; petroleum etherfraction(4compounds),chlorofom(2compounds),ethylacetate(17compounds)andn-butanol(7compounds);ethanol(9compounds) .
- Three dihydrochalcones were isolated from *Albizia.Amara* roots and one dihydrochalcone was isolated from *Acacia mellifera* bark.
- Antibacterial screening against Gram negative bacteria: *Streptococcus mutans* (SM) and *Lacto bacillus* (LB) was conducted and significant results were obtained.
- Different fractions of target species as well as pure compounds were evaluated for their antioxidant potential and significant results were obtained.

## Recommendations

- 1- The targeted species may be investigated for other phytochemicals like alkaloids, tannins, saponins...etc
- 2- Different extracts of targeted species may be evaluated for their anti-inflammatory, anti-malarial and other biological activities.
- 3- A future  $^{13}\text{C}$  NMR may cite additional evidence in favor of the proposed structures for the isolates.
- 4- Two dimensional NMR experiments ( $^1\text{H}$  -  $^1\text{H}$  COSY NMR, HMBC, HSQC) are also recommended for complete elucidation of tentative structures.