3- Results and Discussion

3.1-Phytochemical screening

Seeds of *Acacia nilotica* and fruits of *Vangueria madagascariensis* were screened for different phytoconstituents. A tabulation of the results is presented in Table 3.1.

Table 3.1: Phytochemical screening

Test	*Vangueria madagascariensis*	*Acacia nilotica*
Tannins	+	+
Flavonoids	+	+
Glycosides	+	+
Alkaloids	+	_
Steroids	+	_

3.2. Characterization of isolated compounds

A successive silica gel column chromatography followed by further purification via thin layer chromatography allowed for the isolation of two components from *Vangueria madagascariensis* – compounds I and II. Identification of these compounds was based on extensive UV shifting reagents,IR, ^{1}HNMR and mass spectroscopy data.

3.2.1--compound I

Compound I was isolated from fruits of *Vangueria madagascariensis* as yellow solid.The IR spectrum (Fig.3.1) displayed absorption bands at ν(KBr) 673(C-H , Ar.) ,1105 (C-O) ,1550 , 1645 (C=C , Ar) 1689 (C = O) , 2854 (C- H) aliphatic ,3429cm^{-1} (OH).

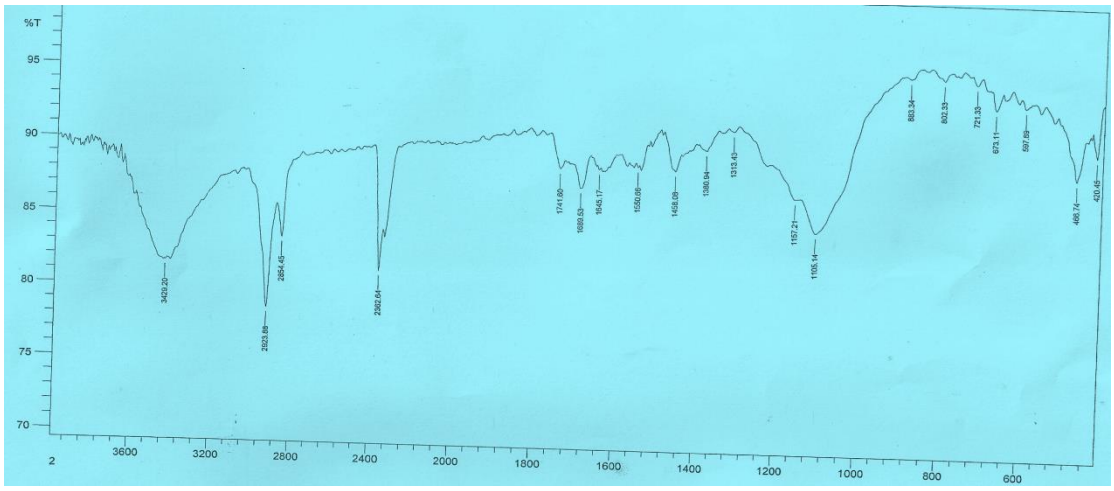


Fig.3.1 : IR spectrum of compound I

The presence of a C=O function excludes the presence of (i)anthocyanins and (ii) catechins .

The U V spectrum (Fig.3.2) showed λ_{max} (MeOH) 273 nm (Fig. 3.2)the presence of band II only indicates that the compound is probably a dihydroflavonol,dihydrochalcone,flavanone or an isoflavone.Among these classes only dihydroflavonols are characterized by a 3-OH function . The sodium methoxide spectrum indicated the presence of a 3-OH function(Fig.3.3).

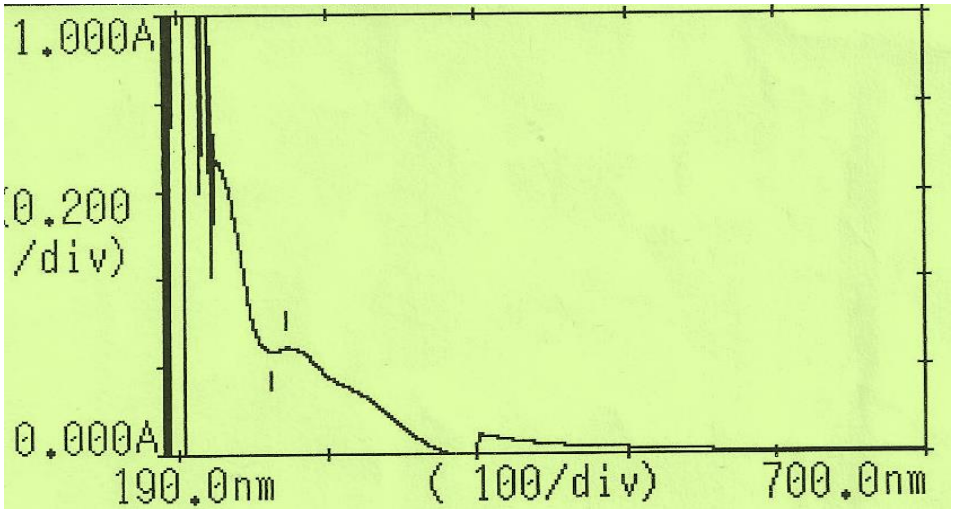


Fig.3.2: UV spectrum of compound I

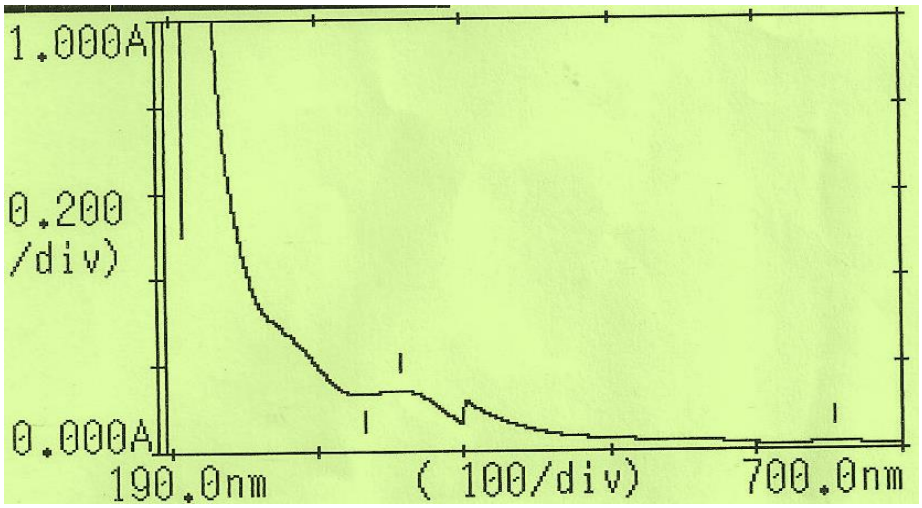


Fig.3.3.: Sodium methoxide spectrum of compound I

The shift reagent sodium methoxide is a strong base capable of ionizing all hydroxyl functions in the flavonoid nucleus , but it is diagnostic of 3- and 4'- OH groups .In both case it gives a bathochromic shift but with decrease in intensity in case 3-OH. Addition of the strong base NaOMe to a methanolic solution of compound I caused(Fig.3.3) a bathochromic shift (84nm) in band I with decrease in intensity. This indicates the presence of a hydroxyl group at C-3.

To investigate the hydroxylation pattern of this isolate , the UV shift reagents were employed .Soudim acetate is a weaker base than sodium methoxide and as such ionizes only the more acidic hydroxyl group in flavonoids i.e. 3-,7- and 4-' hydroxyl groups. Since ionization of 7- hydroxyl group mainly affects band II , sodium acetate is particularly useful diagnostic reagent for the specific detection of 7-hydroxy function .

However, no bathochromic shift was observed in the sodium acetate spectrum(Fig.3.4). This indicates absence of a 7-OH function.

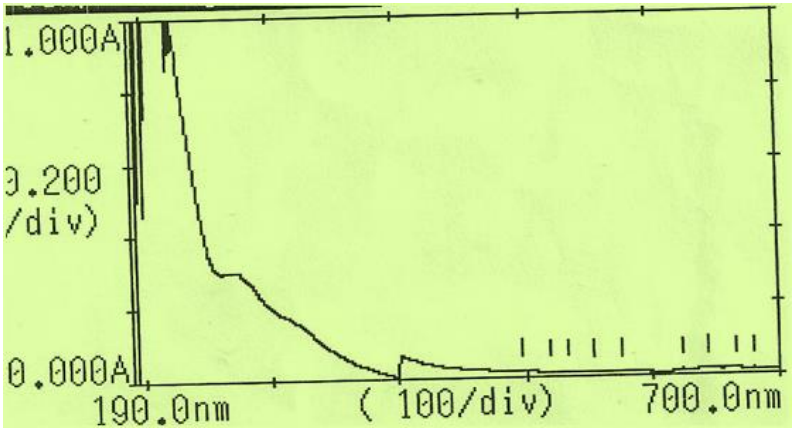


Fig.3.4: Sodium acetate spectrum of compound I

Aluminum chloride chelates with functional groups such as the 5-hydroxy-4-keto-,3-hydroxy-4-keto and ortho dihydroxyl systems and this is evidenced by bathochromic shifts of one or both bands in the spectrum.

$AlCl_3$ complex between the C-4 keto function and either 3- or 5.-hydroxyl group is stable in presence of HCl acid. However catechols yield acid-labile complexes.

The aluminium chloride spectrum did not reveal any bathochromic shift (Fig.3.5) indicating absence of 3- and 5-OH function as well as catechol systems(Fig.3.6).

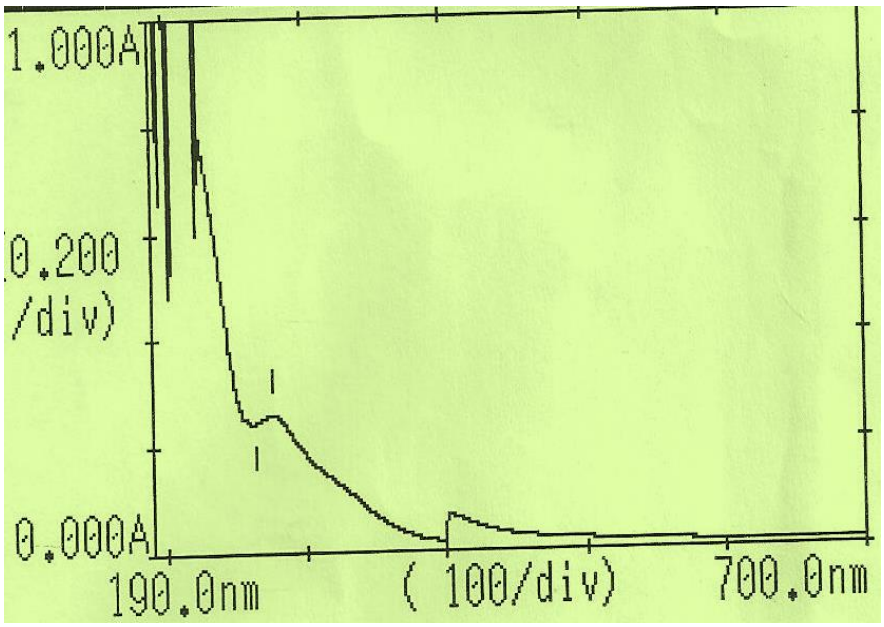


Fig.3.5: Aluminium chloride spectrum of compound I

The ^{1}HNMR spectrum (Fig. 3.6) showed signals for one methyl group at ∂1.23(s,3H) and three acetyl functions at ∂ 1.89 (s,9H). The resonances at ∂6.10 (s,1H) and ∂6.70 was assigned to H-6, H-8 respectively.The C-8 proton usually resonates at lower field relative to C-H due to the deshielding influence of neigh bouring oxygen at position 1. The B-ring protons appeared as singlets at ∂ 6,98 and ∂7.31ppm.

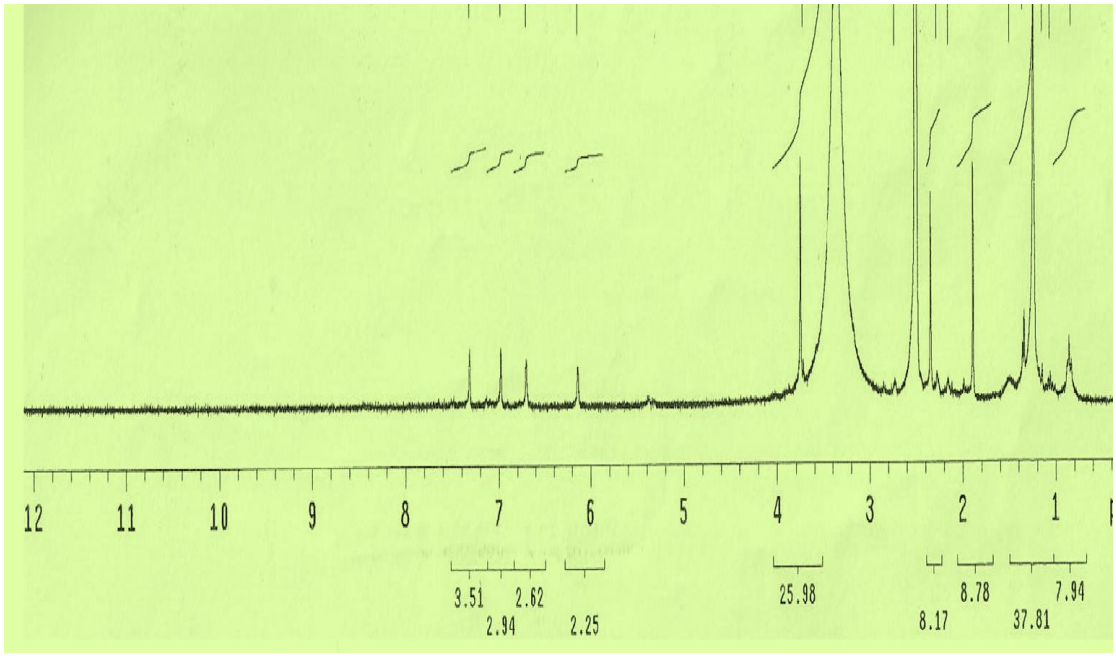


Fig.3.6:^{1}H NMR spectrum of compound I

The ESI-MS(Fig.3.7) showed a molecular ion peak at m/z 381 [M^{+}+H] .Other important fragmentns resulting from retro Diels-Alder fission(Schme 3.1) were shown at m/z 134, 246.Apparantly these intact A and B rings respectively.

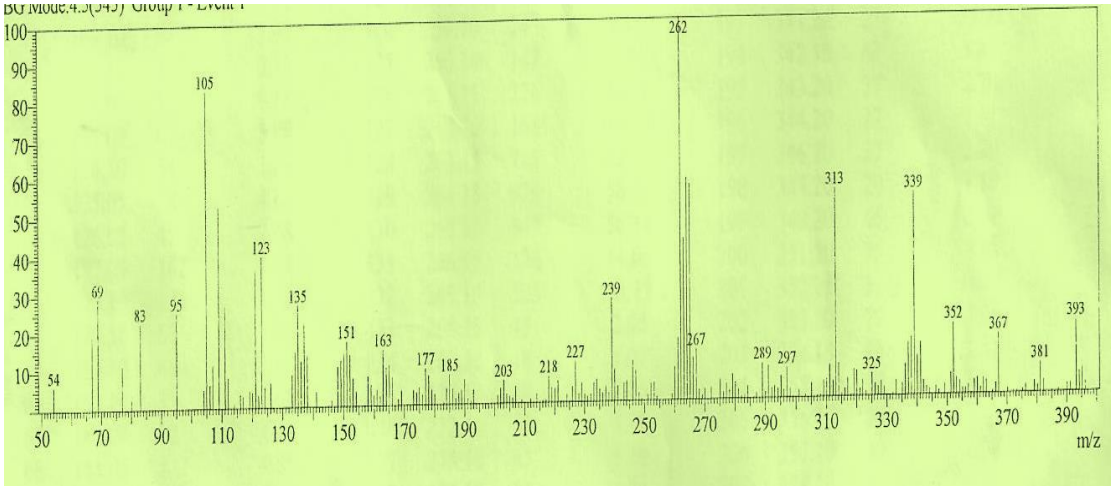
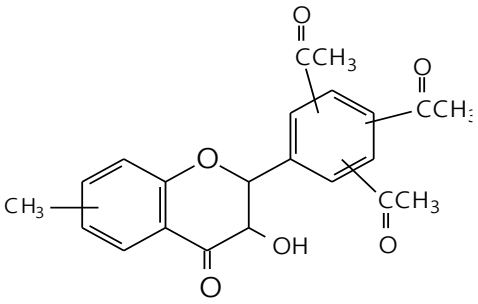
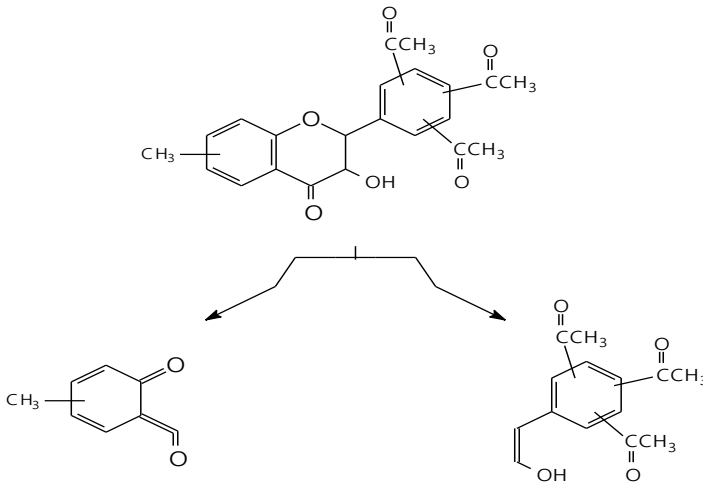


Fig.3.7: Mass spectrum of compound I

On the basis of the above cumulative , the following tentative structure was proposed for compound I.



Compound I



m/z134 m/z246

Scheme 3.1: Retro Diels-Alder fission of compound I

3.2.2. Compound II

The IR spectrum of compound II (Fig.3.7) displayed absorption bands at ν(KBr) : 671(C-H , Ar.) ,1064 (C-O) ,1456 (C=C , Ar) 1739(C = O) , 2923 (C- H) aliphatic , 3353cm^{-1} (OH).

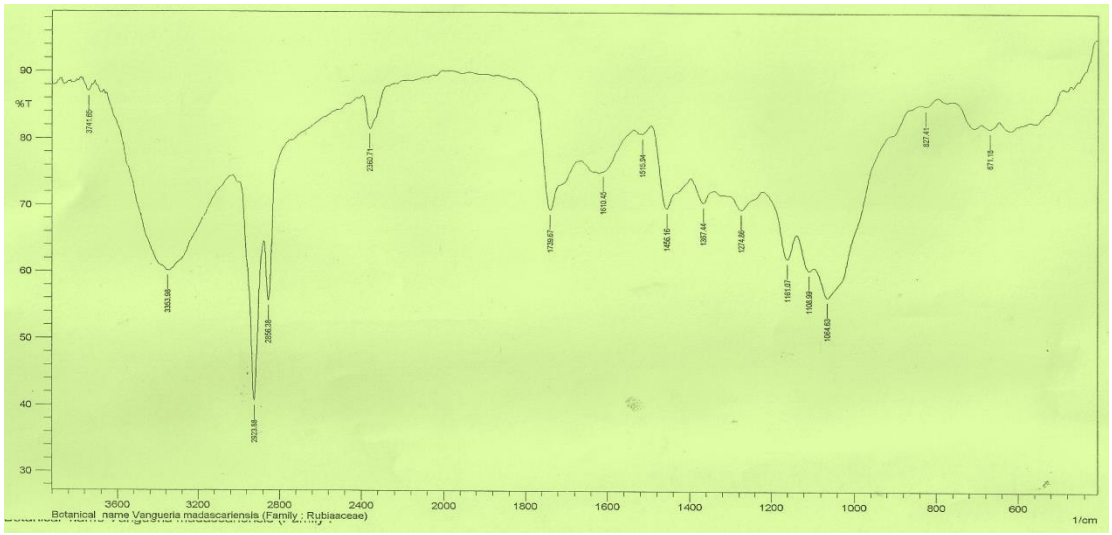


Fig.3.7: IR spectrum of compound II

The VU spectrum (Fig.3.11) showed λ_{max}(MeOH) 232,307 nm.Such absorption is characteristic of flavones.

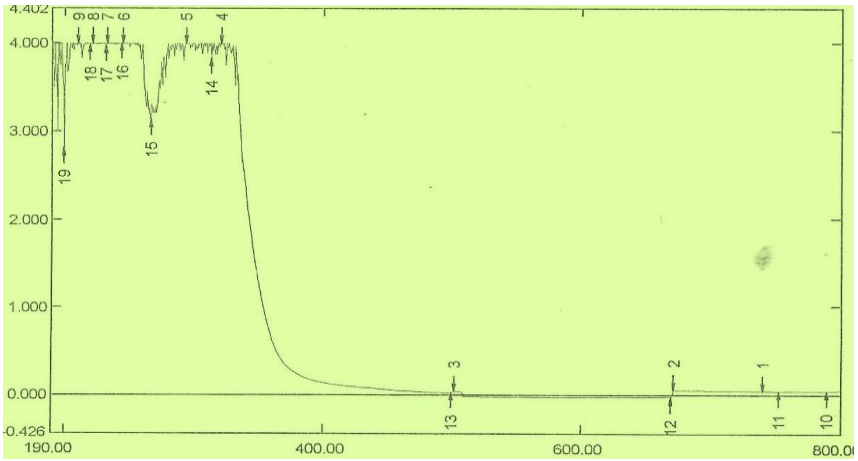


Fig.3.11: UV spectrum of compound II

The sodium methoxide spectrum(Fig.3.12) revealed a bathochromic shift without decrease in intensity and this is diagnostic of a 4`-OH function.

Also, the aluminum chloride spectrum(Fig.3.13) did not reveal a bathochromic shift indicating absence of 3- and 5-OH functions as well as catechol moieties.

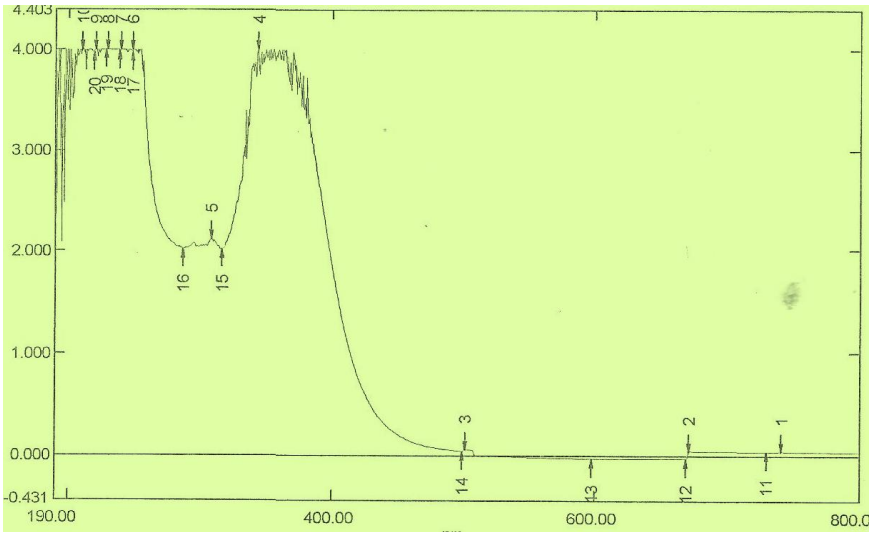


Fig.3.13:Sodium methoxide spectrum of compound II

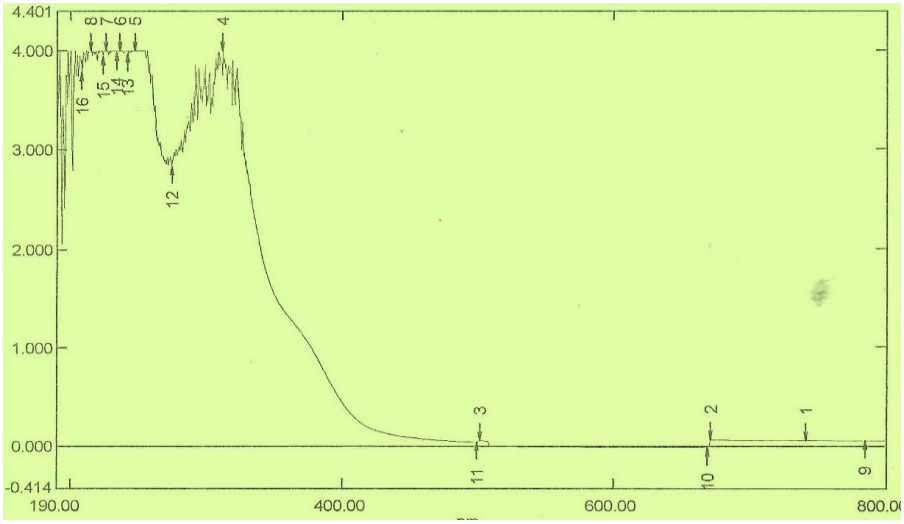


Fig.3.13:Aluminium chloride spectrum of compound II

The ^{1}HNMR spectrum (Fig. 3.14) showed a signal for one methyl group at ∂1.23, an acetyl function at ∂ 2.22 and a methoxyl at ∂3.77ppm . The resonances at ∂6.99 and ∂7.71 were assigned to the aromatic protons of A and B rings respectively.

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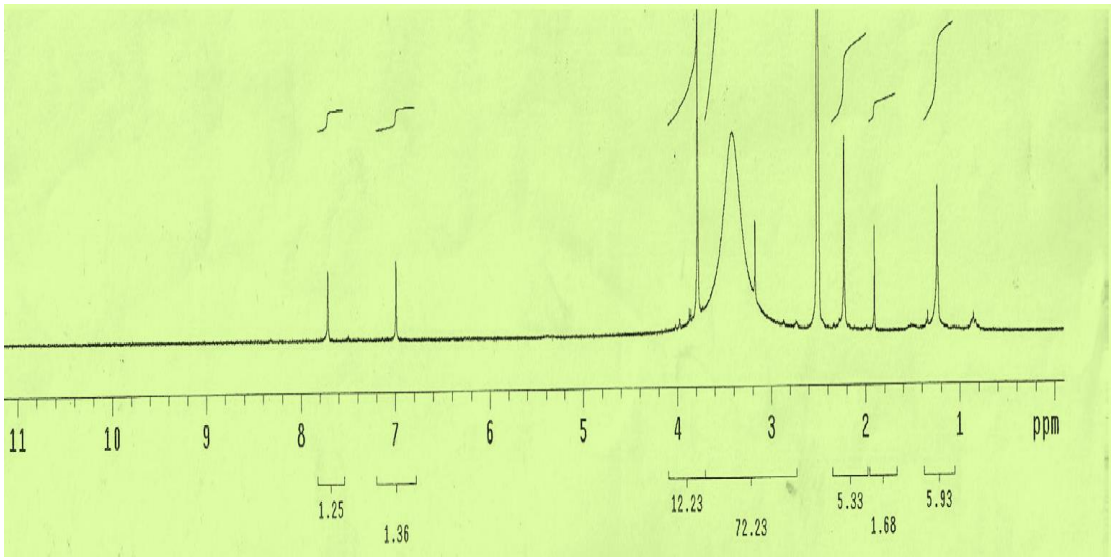


Fig.3.14:^{1}H NMR spectrum of compound II

The ESI-MS(Fig.3.15) showed a peak at m/z323 [M^{+}-H^{+}] .Other important fragmentns resulting from retro Diels-Alder fission(Scheme 3.2) were shown at m/z 150, 175.Apparantly these correspond to intact A and B rings respectively.

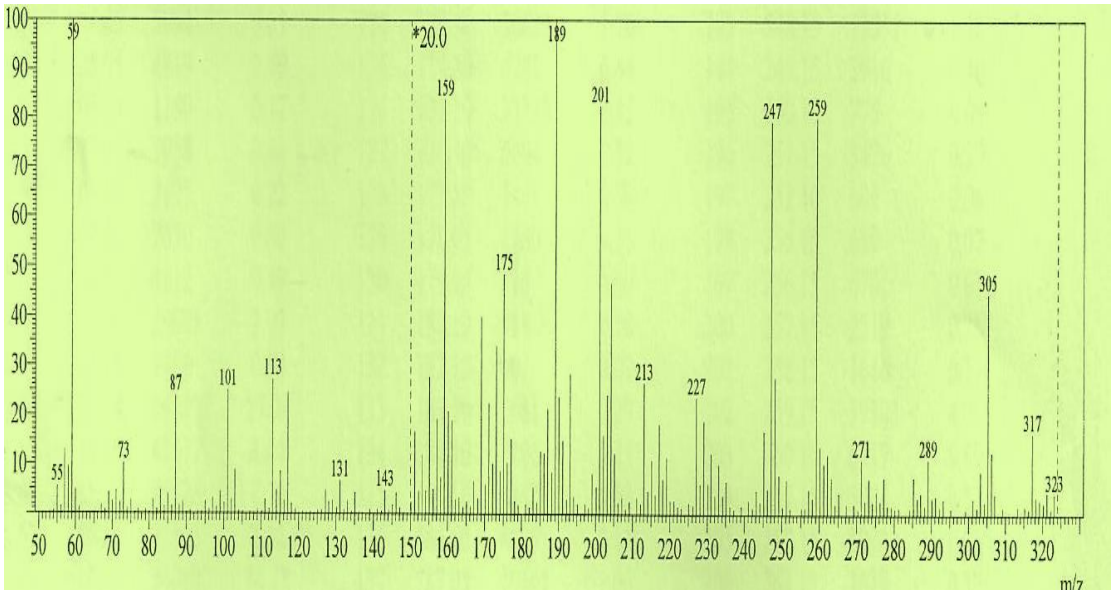
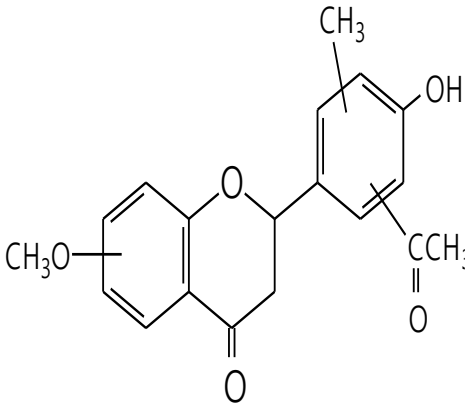
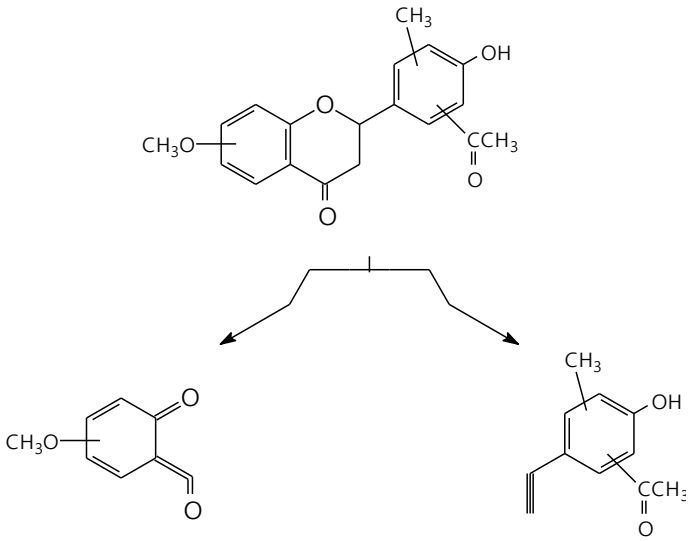


Fig.3.15: Mass spectrum of compound II

On the basis of the above cumulative , the following tentative structure was proposed for compound II.



Compound II



m/z150 m/z175

Scheme 3.2: Retro Diels-Alder fission of compound II

3.4-Antibacterial activity

In cup plate agar diffusion assay, the chloroform fraction of *Vangueria madagascariensis* , compound II and *Acacia nilotica* seed oil were evaluated for their antimicrobial activity.The aqueous extract of *Acacia nilotica* seeds was also evaluated.

The chloroform fraction of *Vangueria madagascariensis* did not show antibacterial activity, but it showed significant inhibitory activity against the fungi: *Candida albicans* and *Aspergantillus niger* . Compounds II showed antifungal activity. However,it did not reveal antibacterial activity(Table 3.3) .The activity is expressed as less active, if the zone of inhibition is 9-12 mm; active : 13-18mm; very active if the inhibition zone is greater than 18mm.

Acacia nilotica aqueous extract showed significant antibacterial activity and significant antifungal activity. *Acacia nilotica* oil also showed antibacterial and antifungal activity, but it was inactive against *Bacillus subtilis*.

Table 3.3 : Antimicrobial activity of studied species

Sample	Conc mg/ml	E.c	P.s	S.a	B.s	C.a	A.s
Compound II	100	_	_	_	_	13	15
Chloroform extract	100	_	_	_	_	17	20
Acacia nilotica aq. extract	100	17	16	18	18	17	15

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

Drug	Conc. mg/ml	B.s.	S.a.	E.c.	P.a.	S.t
Ampicillin	40	15	30	-	-	-
	20	14	25	-	-	-
	10	11	15	-	-	-
Gentamycin	40	25	19	22	21	22
	20	22	18	18	15	17
	10	17	14	15	12	14

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

Drug	Conc. mg/ml	A.n	C.a
Clotrimazole	30	22	38
	15	17	31
	7.5	16	29

- S.a*: Staphylococcus aureus*
- E.c*: Escherichia coli*

- P.a: *Pseudomonas aeruginosa*
- A.n: *Aspergillus niger*
- C.a: *Candida albicans*
- S.t: *Salmonella typhi*
- *B.a: Bacillus subtilis*
- M.D.I.Z: Mean diameter or growth inhibition zone (mm). Average or two replicates, inhibition zone >=15: sensitive, <15: resistant.