

ISOLATION AND CHARACTERIZATION OF SOME FLAVONES FROM *ARUM CYRENAICUM* (ARACEAE)

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ABSTRACT

Information on *Arum cyrenaicum*, which is a common herb in Aljabal Al-Akhdar (Libya), is very scarce. Hence it was planned to investigate the flavonoids of this species which is used in traditional medicine against some human disorders. Phytochemical screening of the whole plant revealed the presence of flavonoids, alkaloids, terpenes, carbohydrates and sterols. Fractionation of the butanol fraction over a polyamide column gave three flavones-compounds I, II and III. The structures of these flavonoids were elucidated via a combination of spectral techniques (UV, ¹HNMR and MS).

KEYWORDS: *Arum cyrenaicum*, Flavonoids, Isolation.

INTRODUCTION

The genus *Arum* which comprises 28 species is native to Europe, Asia and north Africa.^[1,2] *Arum* have been used traditionally for centuries.^[3,4] This genus is divided into two sub-genera *Arum* which contains all the species except *Arum pictum* which belongs to the sub-genera *Gymnomesium*. The toxicity of *Arum* species is well known and most traditional uses concentrate on the anticancer potential of these species.

Kochmarov et al.^[5] reported that *Arum balansanum* and *Arum detruncatum* are used externally for haemorrhoids. In Indian system of medicine *Arum* is used against boils,^[6] while in Jordan it is used as anticancer.^[7] The plant is used in Palestine for prostate disorders.^[8] *Arum* flowers are used for wounds and haemorrhoids. *Arum italicum* is applied topically for warts.^[9,10] This species is used in Italy for CNS disorders and rheumatic pains. It is also used against eczema, cancer and haemorrhoids.^[11,12]

Arum cyrenaicum is a perennial herb in the sub-family Aroideae of the family Araceae. The plant reaches 13-27 cm in height. Information on this plant which is a common herb in Aljabal Al-Akhdar (Libya), is very scarce. Hence it was planned to investigate the flavonoids of this species which is used in traditional medicine against some human disorders.

MATERIALS AND METHODS

Materials

Plant material

Arum cyrenaicum was collected from Aljabal Al-Akhdar region- Libya during the flowering stage. The plant was kindly identified and authenticated by Dr. Naser Elshekhi, lecturer of taxonomy at Botany Dept, Faculty of Science, Benghazi University. A voucher specimen was deposited at the herbarium of faculty of science, Sirt University. The aerial parts of the plant were air dried and powdered.

Instruments

Instruments used in flavonoids measurements include:

- i- UV viewing lamp at the long wavelength.
- ii- UV- Vis spectrophotometer 2401 Shimadzu.
- iii- UVIKON 931 double beam UV- vis. spectrophotometer. All measurements in region of 200-500 nm.
- iv- Bruker NMR spectrophotometer operating at 300 MHz for ¹HNMR in DMSO-d₆.
- v- Mass spectrometer - Finnigan Mat SSQ 7000.

Methods

Phytochemical screening

Powdered whole plant of *Arum cyrenaicum* was screened for major secondary metabolites according to the method described by Harborne.^[13]

Isolation of flavonoidal constituents

Whole plant of *Arum cyrenaicum* (500g) was macerated with aqueous alcohol till exhaustion. Removal of the solvent under reduced pressure gave a crude product. The crude product was dissolved in warm water and partitioned with n-butanol. Paper chromatography of the butanol fraction using Whatmann 3mm irrigated with

20% acetic acid gave the best separation of the flavonoids. A Polyamide column chromatography of the butanol fraction gave three flavonoids: compound I (R_f 0.79), compound II (R_f 0.89) and compound III (R_f 0.58-solvent 20% acetic acid). The polyamide column is summarized in Table (1)

Table 1: Column chromatography of butanol fraction *A. cyrenaicum*.

Solvent	Fractions (25ml)	R_f	Colour in UV		Isolated compound
			NH_3	$AlCl_3$	
Methanol: Water 30:70	10-13	0.79	YG	GY	Compd. I
Methanol: Water 90:10	25-28	0.89	Br.	Br.	Compd. II
Methanol: Water 60:40	20-24	0.58	GY	Y	Compd. III

Paper chromatography: 20% acetic acid; whatmann No. 3mm.

Y.=Yellow

Br.=Brown G.Y.=Greenish-Yellow

RESULTS AND DISCUSSION

The whole plant of *Arum cyrenaicum* was screened for major secondary metabolites and the results are summarized in Table (2).

Table 2: Results phytochemical screening *A.cyrenaicum*.

Constituents	Results
Flavonoids	+
Carbohydrates and / or glycosides	+
Sterols and / or triterpenoids	+
Tannins	-
Alkaloids	+
Saponins	-

Identification of compound I

The UV absorption spectra of compound I (Fig. 1 and Table 3) showed λ_{max} 274, 335nm. Such absorption is characteristic of flavones.^[14,15] It also revealed a bathochromic shift(15nm) in band-I (Fig.2) with sodium methoxide, indicating^[14] the presence of a free OH function at C- 4'. The aluminium chloride spectrum(Fig.3) showed a bathochromic shift(49nm) in band -I relative to methanol spectrum suggesting^[14] the presence of a free OH group at C- 5. The $AlCl_3$ / HCl spectrum showed no hypsochromic shift in band- I relative to $AlCl_3$ spectrum which lends evidence for the absence of a catechol system (Fig.4). The absence of *ortho*-dihydroxy systems is further confirmed through the boric acid spectrum (Fig.5). The sodium acetate spectrum (Fig.6) did not show a bathochromic shift in band- II diagnostic of a 7-OH function.

Table 3: UV data of compound I.

Addition to MeOH	λ_{max} (nm)
None	274, 335
NaOMe	281, 305,350
$AlCl_3$	281, 305, 350, 384
HCl	282, 303, 343
NaOAc	282, 396
NaOAc / H_3BO_3	275, 339, 347

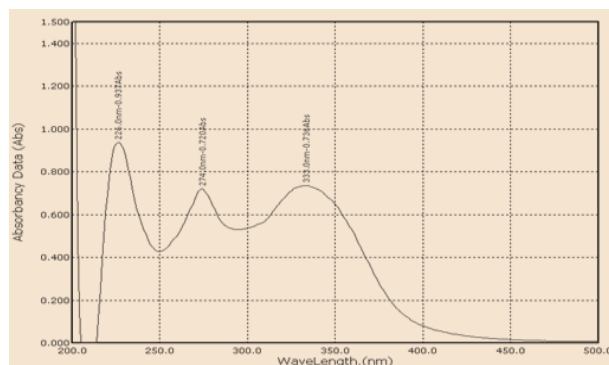


Fig. 1: UV spectrum of compound I.

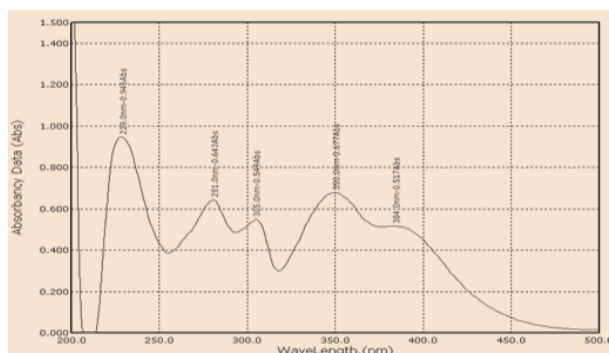


Fig. 2: Sodium methoxide spectrum of compound I.

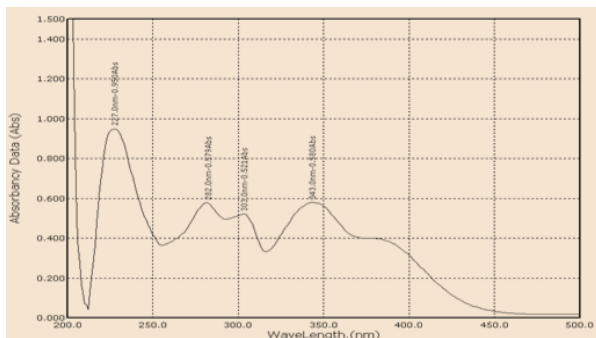


Fig. 3: Aluminium chloride spectrum of compound I.

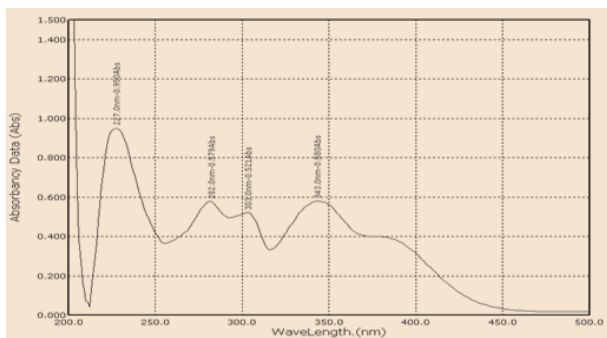


Fig. 4: Aluminium chloride/HCl spectrum of compound I.

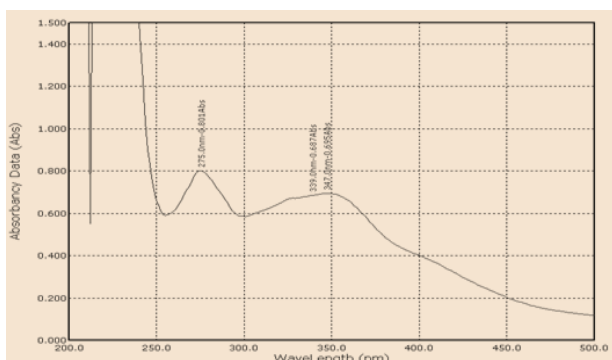


Fig. 5: Boric acid spectrum of compound I.

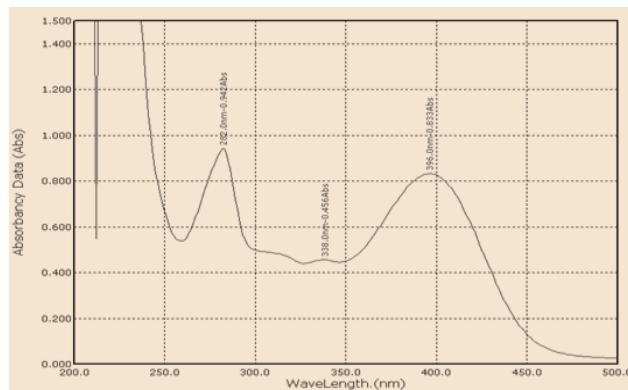


Fig. 6: Sodium acetate spectrum of compound I.

The ¹H- NMR spectrum (DMSO-d₆) (Fig.7) showed signals at δ(ppm): 1.22(rhaminose Me) ; 3.00-3.70(rhamnoglucosyl moiety), 3.95 (MeO) ; 4.85(sugar anomeric proton) ; 6.77(C₆-H) ; 6.93(C₈ -H) ; 7.94,8.08(Ar. protons). The mass spectrum (Fig. 8) gave m/z607 (for M⁺-H).

The above cumulative data is consistent with the published data for chrysoeriol-7-rhamnoglucoside:

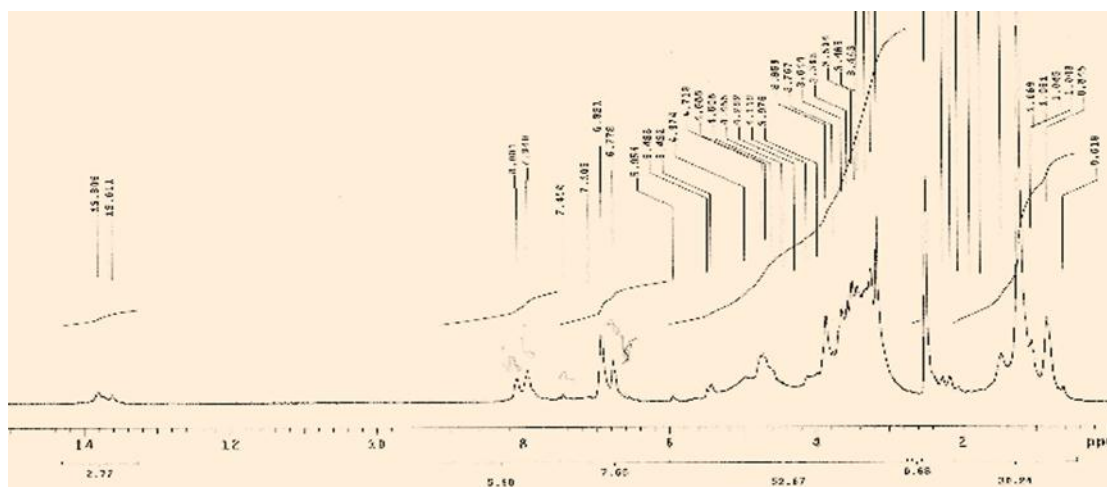
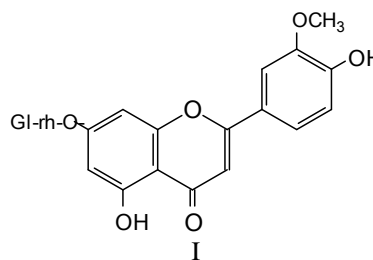


Fig. 7: ¹HNMR spectrum of compound I.

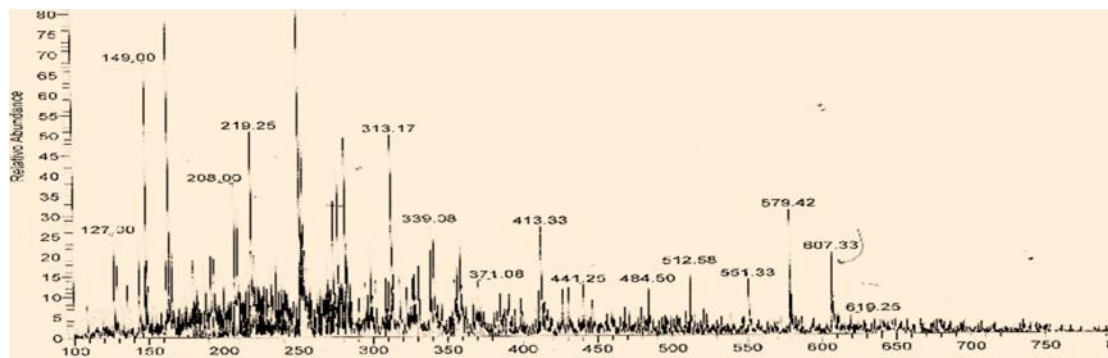


Fig. 8: Mass spectrum of compound I.

Identification of compound II

The UV spectrum of compound II (Fig. 9 and Table 4) showed a pattern characteristic of flavones at λ_{max} 273, 332nm. Addition of the UV shift reagent sodium methoxide revealed a bathochromic shift(Fig. 10) in band-I (71nm) with increased intensity suggesting the presence of a free OH group at C- 4`. The AlCl₃ spectrum(Fig.11) showed a bathochromic shift in band - I (71nm) relative to methanol spectrum.This shift suggests a B-ring catechol system as evidenced by the degeneration of AlCl₃ / HCl spectrum (Fig. 12) .The sodium acetate spectrum(Fig.13) showed a bathochromic shift (8nm) in band - II diagnostic of free 7- OH function.

Table 4: UV absorption spectra of compound II.

Addition to MeOH	λ_{max} (nm)
None	273, 332
NaOMe	283, 336, 403
AlCl ₃	283, 336, 403
HCl	281, 339, 341
NaOAc	281, 339

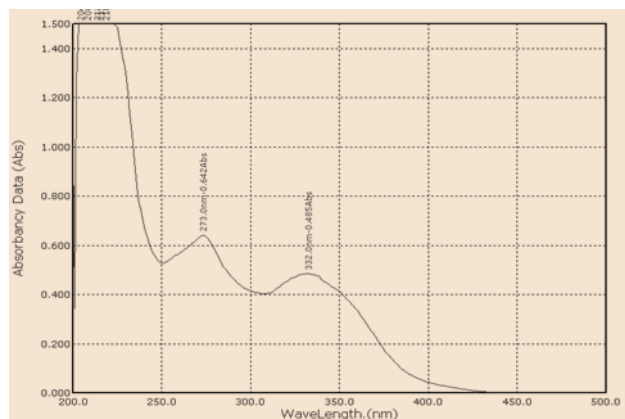


Fig. 9: UV spectrum of compound II.

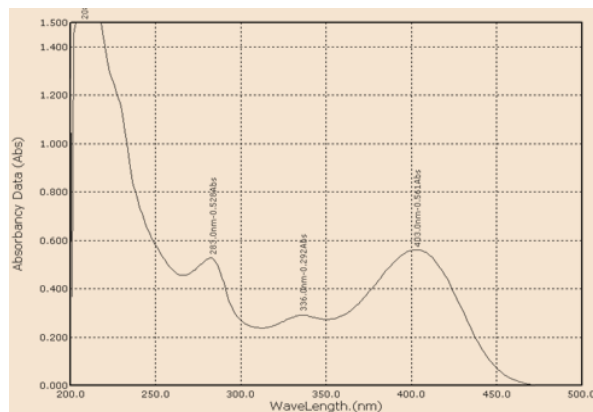


Fig.10: Sodium methoxide spectrum of compound II.

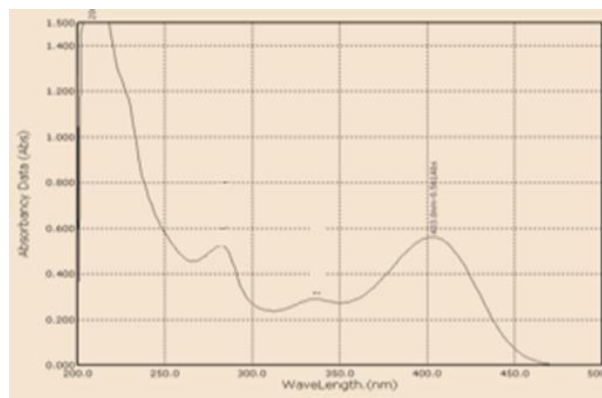


Fig. 11: Aluminium chloride spectrum of compound II.

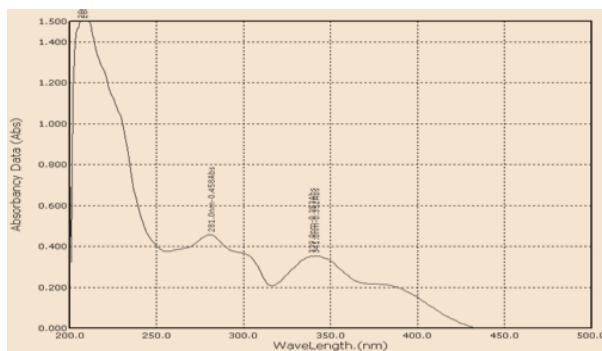


Fig.12: Aluminium chloride/HCl spectrum of compound II.

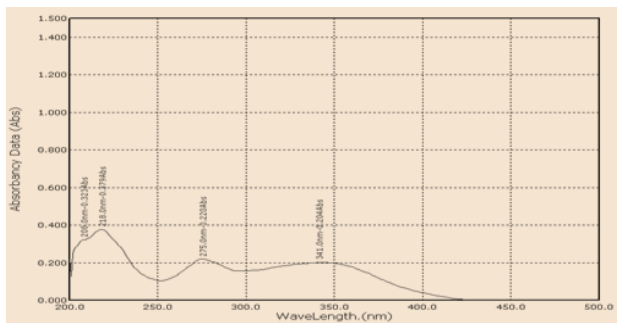


Fig. 16: UV spectrum of compound III.

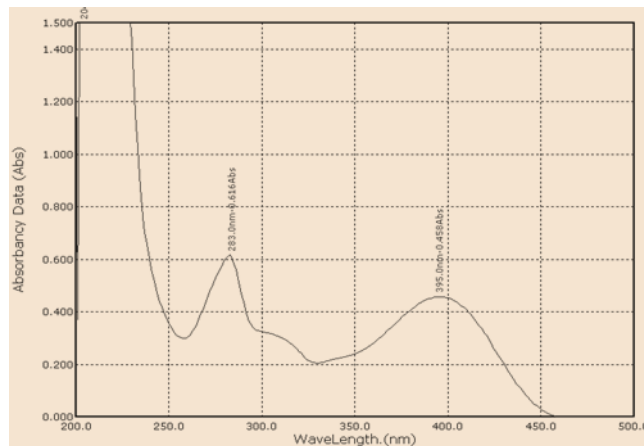


Fig. 19: Sodium acetate spectrum of compound III.

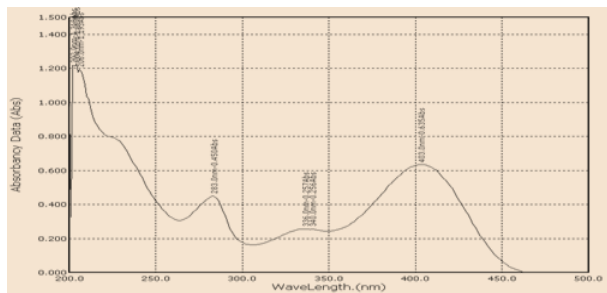


Fig. 17: Sodium methoxide spectrum of compound III.

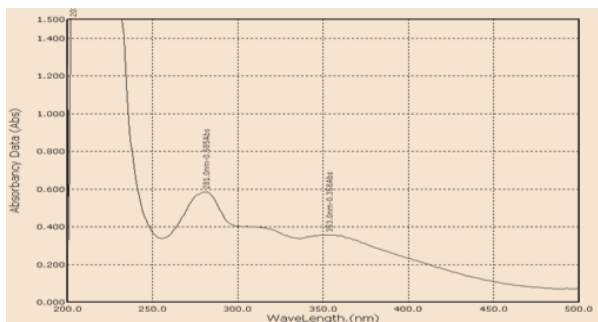
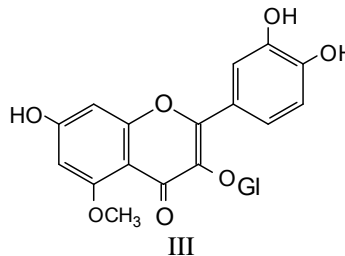


Fig. 18: Boric acid spectrum of compound III.

The ¹H- NMR spectrum of compound III (DMSO-d₆) (Fig.20) showed signals at δ (ppm): 3.87 (MeO); 4.40-5.00(glucose moiety) ; 6.50(C₆-H) ; 6.90(C₈-H) ; 7.85, 8.04(Ar. protons). The mass spectrum(Fig.21) gave m/z479 for M⁺ - 2H (glycoside) and m/z317 for M⁺ - 2H(aglycone).

On the basis of the above spectral data, the following tentative structure was proposed for compound III:



However, a future 2D NMR experiments (¹H-¹H COSY NMR HSQC, HMBC) may lend evidence for the citation of the methoxyl function at C₅ and the location of the glycosidic linkage at C₃.

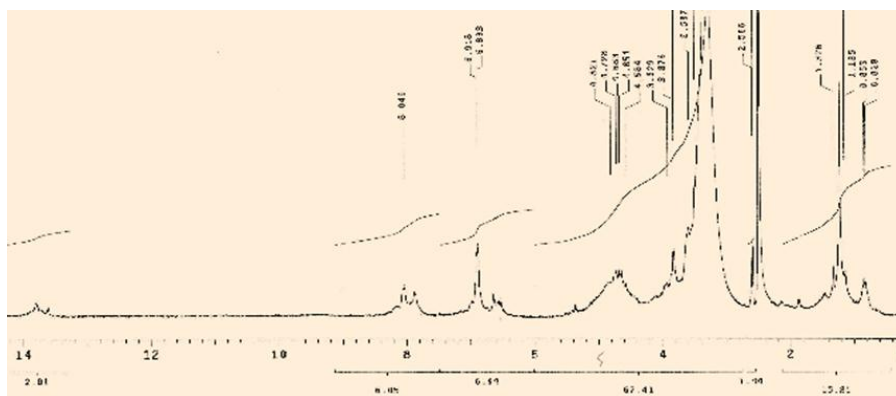


Fig. 20: ¹HNMR spectrum of compound III.

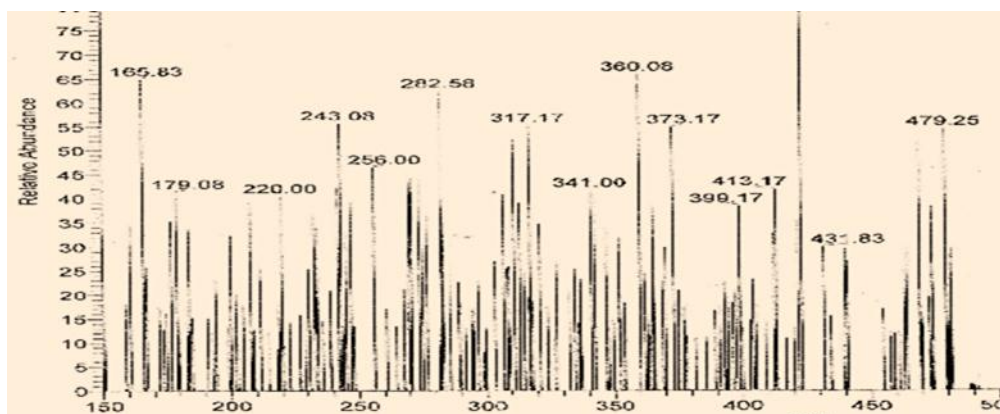


Fig. 21: Mass spectrum of compound III.

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