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# ISOLATION AND CHARACTERIZATION OF BIOACTIVE COMPOUND FROM *Erythrina senegalensis* Stem Bark Extract

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***ABSTRACT***
***Medicinal plants have emerged as some of the most widely studied plants and significant interest has been shown in their chemistry because of their potential application in medicine. Erythrina senegalensis DC (Fabaceae) is a thorny shrub or small tree with common names that include coral tree (English) and mijirya (Hausa, Nigeria). The main reported diseases for which E. senegalensis was used by the traditional healers were amenorrhea, malaria, jaundice, infections, abortion, wound, and body pain (chest pain, back pain, abdominal pain etc).This study aimed at isolation of bioactive compound from the stem bark of Erythrina senegalensis. The stem bark of Erythrina senegalensis was extracted with acetone using soxhlet extraction techniques. The extract was subjected to Thin Layer Chromatography and column chromatography. The fractions obtained were subjected to bioactivity test using agar diffusion technique. Acetone fraction 2 ($ACF_2$fraction) however show growth inhibition against clinical isolates of S. typhi, S. dysentarae, S. aureaus, E. coli, A. niger and C. albicans to significant extent. The fraction was then characterized using GC-MS and IR techniques. The GC-MS data showed the major component at 28.13 minutes retention time identified as bis(2-ethylhexyl)-1,2-benzenedicarboxylate.***
***Keywords: Erythina senegalensis, soxhlet, extraction, characterization***

## INTRODUCTION

There has been a rapid expansion of allopathic health care in Nigeria over the last decade including an increase in the number of allopathic health care providers. At the same time, because the majority of Nigerian uses traditional medicine, the government of Nigeria has shown appreciation for the importance of traditional medicine in the delivery of health care (WHO, 2001).

The practice and the use of traditional medicine and herbs in Nigeria is now coming to the fore since various health workers observed that in Nigeria, 70% to 80% of the populations rely on plants for their primary health care needs. Coming to realize the benefits of natural herbs to synthetic drugs and have remained effective in the fight against microbial infections (Olukoya *et al.,* 1993).

*Erythrina senegalensis* (known as Mijiriya in Hausa)was used for the treatment of malaria, jaundice, infections, gastrointestinal disorders (gastric ulcer, diarrhea, constipation) amenorrhea, dysmenorrheal, sterility, onchocerchosis, body pain (chest pain, back pain, abdominal pain headache and body weakness). The plant was also reported to have wound healing and contraceptive properties (Adiaratou**,**2008).*Erythrina* bark is used to treat rheumatic joint pain, including back and knee pain. It is said to promote urination, reduce edema, and relieve itching skin. It is also used to kill parasites, relieve toothaches, dispel wind and dampness, and to generally unblock the body's meridians, or energy channels. It has anti-fungal properties, and is used to treat dysbiosis (yeast overgrowth or cadidiasis) (Mao, 2010). The stem bark of *Erythrina senegalensis*is traditionally used by the Bamun population (Western Cameroon tribe) against liver disorders as a decoction (Jean, *et al.,* 2008).This research therefore aimed at isolation and characterization of the bioactive compound from the stem bark of *Erythrina senegalensis.*

## METHODS

### Sample Collection and Identification

The stem bark of the plant was collected in Biu, Borno state and was identified in the Department of biological science, Abubakar Tafawa Balewa University, Bauchi. The sample was air dried under shade and crushed into fine powder using mortar and pistil

### Extraction

Crude acetone extract of *Erythrina senegalensis* stem bark was achieved using Sohxlet extraction techniques (Laurence and Christopher, 1992). The extract was then concentrated using rotary evaporator, dried in an oven then subjected to thin layer chromatography and column chromatography for further isolation and purification.

### The isolation and purification of the bioactive compounds

### Thin layer chromatography (TLC)

Thin layer chromatography (TLC) was carried out as described by James *et al.,*(1987).In current study, TLC was performed on precoated silica gel aluminium plate and the retarding factor (Rf) values determined based on spots (Azra, *et al*., 2012).

The extract was applied as a small spot (1.0 to 2.0 mm diameters) of solution about 1.0 cm from the end of the plate opposite the handle. As small a sample as possible was used, since this will minimize tailing and overlap of spots; the starting position was indicated by making a small mark near the edge of the plate (base line).

A developing solvent (mixture of solvents determined by trial) was poured into the TLC tank to a depth of a few millimeters. The atmosphere in the chamber was then kept saturated by keeping the tank closed all the time except for the brief moment during which a plate was added or removed.

The spotted plate was then placed in the TLC tank and allowed to develop in the adsorbent by capillary action. When the solvent reached the front, the plate was removed from the tank, the position of the solvent front was marked, and the solvent was allowed to evaporate. The spots were visualized with iodine vapor.

The relationship between the distance travelled by the solvent front and the spots was expressed as Rf value (eqn 1) and is strongly dependent upon the nature of the adsorbent and solvent.

$$Rf = \frac{Distancetravelbycompanent}{distancetravelbysolvent} \text{.....eqn 1}$$

### Column chromatography

It was carried out as described by Donard *et al.,* (1988). The solid stationary phase silica gel was packed in the column tube using n-hexane in making the slurry. When the column was loaded, the stopcock was turn on to allow the solvent level to drop to the top of the packing, but do not allow the solvent layer to go below this point.

When the packing was completed, the solution of the sample was then loaded to the top of the column using a pipette without disrupting the flat top surface of the silica gel. A small layer of protective white sand was added to the top of the sample and eluting solvent (ethyl acetate/acetone, 1:1, v/v) was continuously added while small (20 $cm^3$ -25 $cm^3$) fractions were collected at the outlet of the column tube simultaneously. Once all the materials have been removed from the column tube, each fraction was evaporated and dried. The fractions were then monitored using TLC to ascertain single compound in the fractions.

### Biological Activity Test of the Isolated Compound

Agar disc diffusion method was adopted as described by Ramzi, *et al.,* (2009). Alo, *et al.,* (2012). Abbas, *et al., (*2016) and Cleidson, *et al.,* (2007)

The powdered agar media was mixed with water and steamed to dissolve the agar, the whole were then sterilized in an autoclave at 121$^{o}$C and subsequently allowed to cool to about 45$^{o}$C (a temperature at which the agar remains molten). Some 15-20 ml of the molten agar media were then poured into the sterile labeled Petri dishes and were left undisturbed until the agar sets. The plates were allowed to cool and kept upside down in a refrigerator at 4$^{o}$C.

The antibacterial activity of the isolated compound was assessed against *Salmonella typhi, Salmonella dysentarae, Escherichia coli, Shigella aureaus, Trichophyton rubrum, Aspergillus niger* and *Cadida albicans* maintained in brain heart infusion (BHI) at 20ºC. 300 $cm^3$ of each stock–culture were added to 3 $cm^3$ of BHI broth. Overnight cultures were kept for 24 h at 36ºC ± 1ºC and the purity of cultures was checked after 8 hrs of incubation. After 24 hrs of incubation, bacterial suspension (inoculum) was diluted with sterile physiological solution, for the diffusion tests, to $10^8$ CFU/$cm^3$ (turbidity = McFarland). The bacterial inoculum was uniformly spread using sterile cotton swab on a sterile Petri dish of the agar. Samples were dissolved and diluted with dimethylsulfoxide (DMSO)/water ($H_2O$) to a concentration of

200mg/cm$^3$.Filter paper discs were impregnated The discs were allowed to remain at room temperature until complete diluent evaporation and kept under refrigeration until ready to be used. Discs loaded with extract fraction were placed onto the surface of the agar in the Petri dishes. Commercial Ciprofloxacin and Amphotericin B. (200 mg) impregnated on a paper were used as control. Tests were performed in triplicate. The disc/plates were incubated at 37°C for 48 h.

The microbial activities of the extracts and controls against selected bacterial strains were recorded as zone of inhibition measured in millimetre with plastic meter rule.

The Student's T–test was used to compare results between the inhibition zones of the sample and that of the standard drugs. P-values lower than 0.05 ($p < 0.05$) were considered significant.

**Fourier Transform-Infrared Spectroscopy (FT-IR)**

A small quantity of the sample was grin with potassium bromide to very fine powder (to remove scattering effects from large crystals). This powder mixture is then pressed in a mechanical press to form a translucent pellet (a special device to allow the sensor to shine through it) through which the beam of the infrared can pass (Geoffrey, 2012).

**Gas Chromatography - Mass Spectrometry (GC-MS)**

Gas Chromatography-Mass Spectrometer model QP5050A SHIMADZU was used for analysis. The column size is 30 meter x 25mm ID x 25µm film thickness. The injection volume was 0.5nµL, injection temperature 250$^o$C and interface temperature is 250$^o$C. The auto-sampler injects 0.5 µl of the sample, injection in un-split mode. The carrier gas was helium, at a working constant flow rate of 1.5 mL/min. Mass spectra were recorded in electron impact mode at 70 eV; electron multiplier 2500V; ion source. Mass spectra data were acquired in the scan mode in m/z range 40-350 uma.

## RESULTS

**Chromatographic Separations of Acetone Extract**

The acetone extract on eluting on column chromatography with ethyl acetate/acetone, 1:1 (v/v) affords a fraction $ACF_2$ among others. This fraction on monitory on TLC shows a spot with Rf value of 0.54 and 0.75 with n-hexane/acetone, 2:5 (v/v and ethyl acetate/acetone, 1:1(v/v) respectively (table 1).

**Table 1: TLC Analysis of $ACF_2$ fractions**

| Solvent mixture | Rf value |
|---|---|
| Hexane/acetone, 2:5(v/v) | 0.54 |
| Ethylactate/acetone, 1/1(v/v) | 0.75 |

Key: $ACF_2$ = Acetone Fraction 2

**Antimicrobial Activityof $ACF_2$**

The biological activity test carried out with the acetone fraction ($ACF_2$) of *Erythrina senegalensis* stem bark shows growth inhibition against *S. typhi, S. dysentarae*, *S. aureaus, E. coli, A. niger* and *C. albicans* to some extent compared with standard drugs ciprofloxacin and amphotericin B. (Table 2).

**Table 2: Antimicrobial Activities of $ACF_2$**

| | Zones of inhibition (mm) | | |
|---|---|---|---|
| | | Control | |
| **Microorganism** | **$ACF_2$** | **cipro** | **distilled water** |
| *S. typhi* | 18 | 23 | 00 |
| *S. dysentarae* | 16 | 24 | 00 |
| *E. coli* | 13 | 26 | 00 |
| *S.aureaus* | 11 | 27 | 00 |
| | | **amp** | |
| *T. rubrum* | 9 | 25 | 00 |
| *A. niger* | 10 | 24 | 00 |
| *C. albicans* | 15 | 23 | 00 |

NOTE: Zone of inhibition ≥ 8 mm is sensitive while < 8 mm is resistant

KEY:$ACF_2$= Acetone fraction 2

*S. typhi = Salmonella typhi* *S. dysentarae = Shigella dysentarae*

*E. coli = Escherichia coli* *S. aureaus = Staphylococcus aureaus*

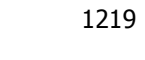
*T. rubrum = Trichophyton rubrum* *A. niger = Aspergillus niger*

*C. albican = Candida albicans* amp = Amphotericin B (antifungal)

Cipro = Ciprofloxacin (antibiotic)

The result of the IR analysis of $ACF_2$ is shown on table 3**.** The result reveals the presence of carbonyl ketone/acid, aromatic C=C, and orthodisubstituted benzene bonds absorption bands at 1709 $cm^{-1}$, 1511 $cm^{-1}$ and 727 $cm^{-1}$ respectively (table 3). While the GC-MS analysis of the same sample reveals a major peak at a retention time 28.13 min with m/z value of 149 (table 4).

**Table 3: FTIR Spectra of $ACF_2$ Fraction from Acetone Extract of *Erythrina Senegalensis* Stem Bark**

| Absorption bands in $cm^{-1}$ | Vibration mode | Functional group |
|---|---|---|
| 2921 | C-H stretching | $CH_3$ |
| 2855 | C-H stretching | $-CH_{2-}$ |
| 1709 | C=O stretching | carbonyl ketone/acid |
| 1612 | C=C stretching | Phenyl, conj. C=C |
| 1511 | C=C stretching | an aromatic |
| 1441 | C=C stretching | Homocyclic aromatic |
| 1369 | C-H def | Alkane |
| 1219 |  stretching | aromatic ester |
| 1169 | C-O-C stretching | . Aliphatic ether |
| 1074 | C-H in plane bending | Aromatic |
| 832 | C-H def | Benzene ring |
| 727 | Ortho disubstituted benzene | Aromatic |

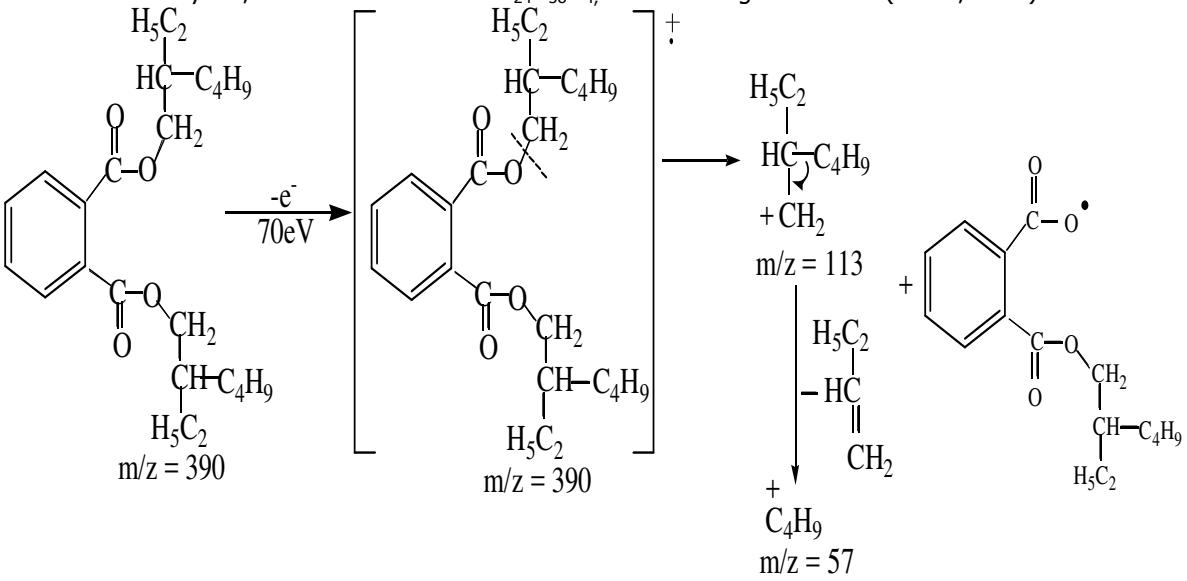
**Note:** Prominent peak at 3349 which represent OH stretching may be due moisture other impurities.

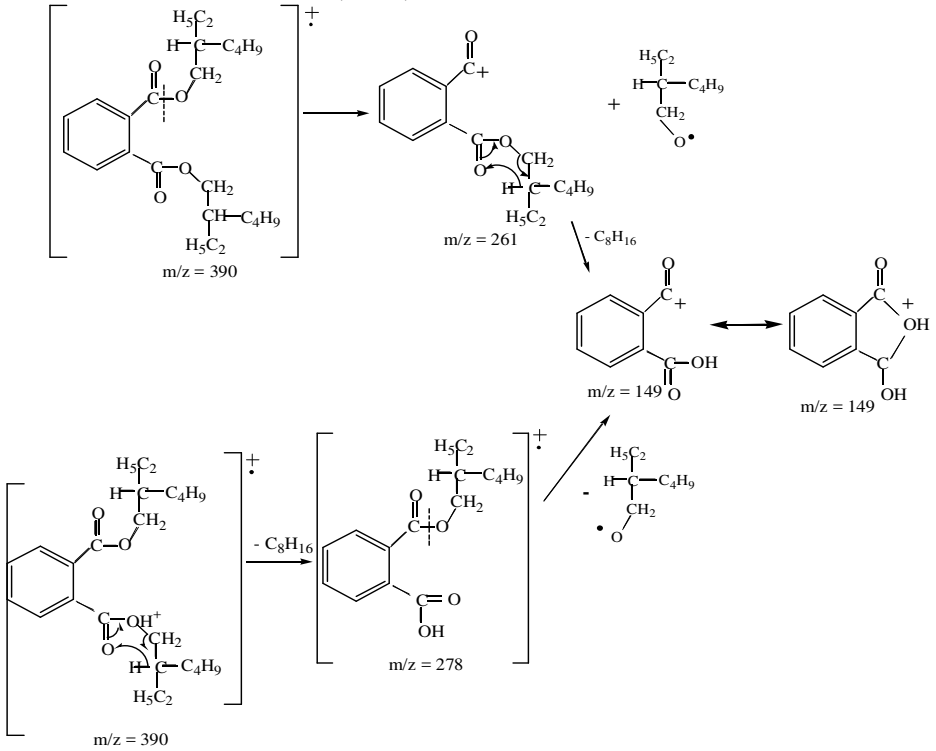
**Table 4: Results for the GC-MS Analysis of $ACF_2$.**

| Spectrum line | GC retention time (min) | Proposed compound | Masses of fragment ions m/z (% abundance of base peak) |
|---|---|---|---|
| Line 34 | 28.13 | Bis(2-ethylhexyl)-1,2-Benzenedicarboxylate | 279, 261, 149(100), 113, 71, 57 |

**Proposed compound from GC-MS spectra of $ACF_2$ fraction**

GCMS data revealed RT = 28.130; peak area = 60.04%; height = 46.02, m/z =149 (base peak). Correspond to Bis(2-ethylhexyl)-1,2-Benzenedicarboxylate as compared with the database of National Institute Standard and Technology (NIST) 08 spectral library collection). Bis(2-ethylhexyl)-1,2-Benzenedicarboxylate, Molecular formular = $C_{24}H_{38}O_4$, Formula weight = 390.56(OECD, 2005).






**Scheme I:** Proposed fragmentation pattern of 1,2-benzene dicarboxylic acid, bis(2-ethylhexyl) ester. A missing molecular ion was observed; follow the arrangement of fragment ions.

## DISCUSSION

### Antimicrobial assay

The fractions $ACF_1$ shows antimicrobial activity against *S. typhi, S. dysentarae*, *S.aureaus, E. coli, A. niger* and *C. albicans* to significant extent compared with standard drugs ciprofloxacin and amphotericin B.

Bis (2-ethylhexyl)-1,2-benzenedicarboxylate has been isolated from stem bark of *Mangifera indica,* this compound inhibits *E. coli, S. aureus, P. aeruginosa, B. subtilis, S. typhi* at minimum inhibitory concentrations MICs of 1.3, 1.4, 1.0, 2,5 and 2.0mg/ml, respectively(Ruchi, *et al*., 2014). Fadipe, *et al*., (2014) also report the isolation of Bis (2-ethylhexyl)-1,2-benzenedicarboxylate from the unripe fruit of *N. latifolia.*and found that the compound can be used in the treatment of infections caused by *Bacillus subtilis* and *Staphylococcus aureus*.

Compound in the GC-MS spectrum of$ACF_2$(RT = 28.13 minutes percentage area peak 60.04%, height 46.02 and intensity 10, 543,016) correspond to 1,2-benzenedicarboxylic acid, bis (2-ethylhexyl) ester (also known as di (2-ethylhexyl) phthalate, DEHP) according to the database of National Institute Standard and Technology (NIST) 08 spectral library collection.

The IR spectrum of $ACF_2$ justify the identification of the named compound Bis (2-ethylhexyl)-1,2-benzenedicarboxylate for there were characteristics absorption of an ester C=O stretching (1709 $cm^{-1}$), phenyl conjugated C=C stretching (1612 $cm^{-1}$), ester C-O-C stretching (1169 $cm^{-1}$) and C-H stretching of disubstituted benzene (727 $cm^{-1}$).

The natural occurrence of Bis (2-ethylhexyl)-1,2-benzenedicarboxylate in a wide variety of plants is already in the literature (Rasika, *et al., 2015;* save*et al., 2015* and Azra*et al.,* 2012). Bis (2-ethylhexyl)-1,2-benzenedicarboxylate are known as curative drugs, antifungal, antitumor, anticancer, antidiabetic, antimalarial (Azra *et al.,* 2012). Sastry and Rao (1995) reported the isolation of bis (2-ethylhexyl)-1,2-benzenedicarboxylate from a marine alga, *Sargassum weightii*, and found to have antibacterial effect on a number of bacteria. Save *et al., (*2015) isolated Bis (2-ethylhexyl)-1,2-benzenedicarboxylate from the *T. peruviana* twigs and that it exerted positive anticancer effects on the prostrate, breasts, colon, lungs, and pancreatic human cancer cell lines by inducing the loss of activity in most cancer cell lines. The presences of Bis (2-ethylhexyl)-1,2-benzenedicarboxylate have been reported from variety of plants including; *Ricinus communis*Linn. (*Euphorbiaceae*) (Sani and Pateh 2009), *Ehretialaevis* (Rasika, *et al., 2015*), *Ocimum americanum* L. (Lamiaceae) (Shubhangi, 2016) and in honey (Monika and Kamaljit, 2016).

## CONCLUSION

Acetone extracts of *Erythrina senegalensis* stem bark were subjected to thin layer chromatography and column chromatography.The bioactivities of acetone fraction ($ACF_2$) shows growth inhibition of *S. typhi, S. dysentarae*, *S.aureaus, E. coliA. niger*and*C. albicans* to appreciable extent compared with standard drugs ciprofloxacin and amphotericin. The GC-MS analysis reveals the presence of bis(2-ethylhexyl)-1,2-Benzenedicarboxylate along with some other compounds in small quantity.

Despite the limitations of the present study, the results shows that bis(2-ethylhexyl)-1,2-Benzenedicarboxylate have been isolated from *Erythrina senegalensis* stem bark. The associations this compound in the *Erythrina senegalensis* stem bark justify the antioxidant, anticancer and antimicrobial reported earlier.

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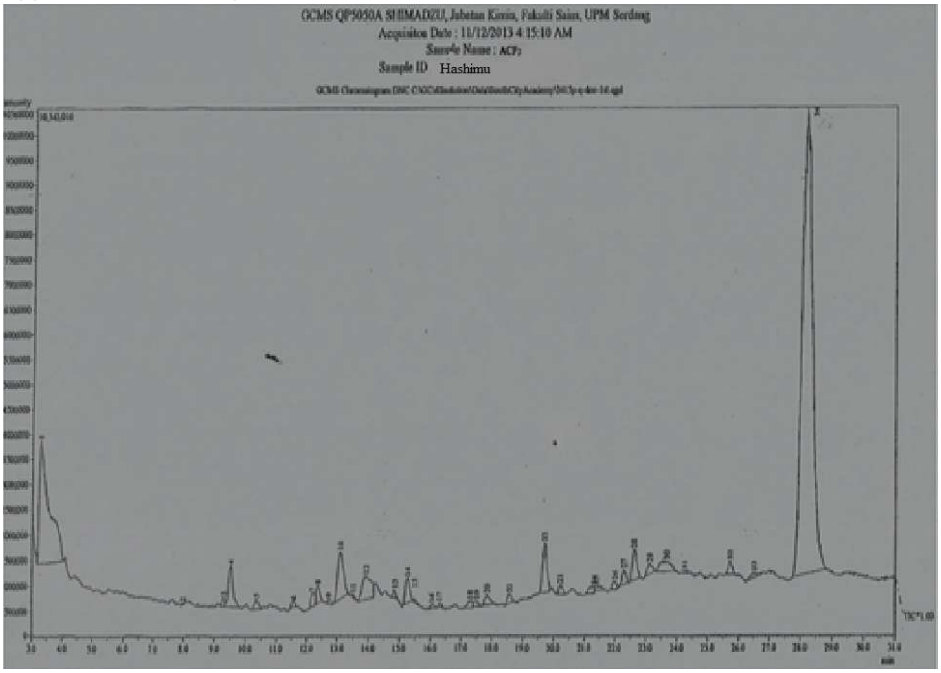
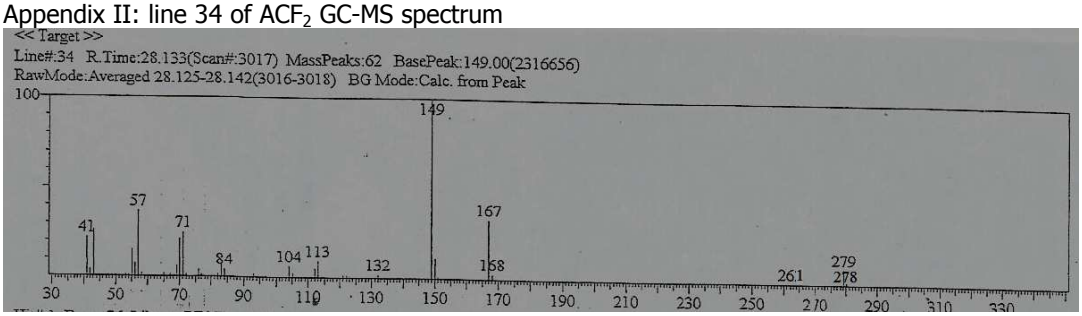
Appendix I: GC-MS spectrum of $ACF_2$




Appendix II: line 34 of $ACF_2$ GC-MS spectrum




Appendix: RF-IR III spectrum of $ACF_2$