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# Isolation and bioactivity of pentacyclic triterpenoid (Betunilic acid) from the bark of *Sarcocephalus latifolius* (Smith Bruce)

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**Abstract**
Sarcocephalus latifolius is a plant variously used in Ethnomedicinal practices in Africa. The dried stem bark of this plant was subjected to continuous extraction using various solvents. The methanol fraction was subjected to vacuum liquid chromatography (VLC) to obtain SB-C2. The structure was established by various spectroscopic studies and comparison of the available data and seen to be Betunilic acid. The bioactivity of this compound was carried out using some clinical pathogens and the activity compared with a standard drug. It was discovered that the compound is comparable to the standard drug.
**Keywords:** Sarcocephalus latifolius, Methanol stem bark, Rubiaceae, vlc, SB-C2, Betunilic acid

## 1. Introduction

*Sarcocephalus* is a genus of tropical evergreen trees and shrubs belonging to the Rubiaceae family. This plant family is one of those frequently used by Ethnomedicinal practitioners in Sierra Leone and neighboring countries. It is a shrub or small spreading tree that is widely distributed in the Savannah and tropical Africa. It is known by the Hausa as *Tafashiya* or *tuwon biri,* known and called *Ubuluinu* in Igbo language in Nigeria. The current folk medicinal applications of *Sarcocephalus latifolius are* varied and numerous, for example the bark and the root extracts are said to be useful in malaria treatment (Akubue and Mittal 1982, *Oye 1990)*. It is used as a tonic and remedy for treating fever, toothaches, dental cures, septic mouth, and diarrhea and in dysentery treatment and also used as chewing stick (Etkin et al 1990, Lamidi et al 1995).The bark is said to be useful in the treatment of wounds, cough and gonorrhea in Nigeria (Madubunyi, 1995).
The leaves are claimed to be useful in the treatment of fever while the roots and bark are claimed to be useful in the treatment of venereal disease, wounds and as odontalgic remedy. *(Pedro and Antonio, 1998).*
The crude root extract had been reported to have antihypertensive effect (Nworgu, 2009).New Indole alkaloids, 21-O-methylstrictosamideaglycone, Augustine, nauclifine, augustidine, 19-0-ethylaugustoline, naucleidinal, 19-epi-naucleidinal, quinovic acid-3β –O- β-D-fucopyranoside, quinovic acid-3β –O- β-D-rhamnopyranoside, scopoletin, and β-sitosterol had been isolated from the root. The strictosamide isolated from it was reported to show moderate antiplasmodial activity against plasmodium falciparium (Pedro and Antonio, 2001)

## 2. Plant Materials:

The stem bark of *Sarcocephalus latifolius* were collected from Okene local Government Area of Kogi state, Nigeria in March 2010.These were identified at the Herbarium of the Department of Biological Sciences of the Faculty of Sciences, Ahmadu Bello University, Zaria, Nigeria. A voucher number was deposited at the herbarium. The sample was air-dried and pulverized using wooden pestle and mortar. Finally, these were stored in an air-tight polythene bag and kept away from moisture until needed for analysis.

## 3. Phytochemical analysis

The plant part was screened for plant metabolites using the pulverized materials. Standard techniques of Brain and Turner (1975) were employed in the phytochemical screening. The presence or absence of the following metabolites were screened; carbohydrates, glycosides, Anthraquinones, cardiac glycoside, saponins, Flavonoids tannins, saponins and alkaloids

## 4. Extraction

Continuous extraction was used for 221.80g of the pounded stem bark of the plant material using the soxhlet extractor. Petroleum ether was initially used to defat it followed by chloroform, ethyl acetate and finally methanol. Each of the extracts was concentrated *in vacuo* using rotary evaporator at 40 $^0$C and the resultant crude samples were air-dried until constant weight were obtained to afford; Petroleum ether extract (2.40g or 1.08%) ,Chloroform extract

(0.47g or 0.21%),Ethyl acetate extract(0.9g or 0.41%) and Methanol extract (22.7g or 10.23%) of crude products respectively.

**5. Isolation of SB-C2 from methanol fraction**

3g of the methanol extract of the bark of *Sarcocephalus latifolius* was dissolved in methanol and filtered to remove any undissolved lump. This was pre-absorbed in sufficient celite. This was packed with 30g silica gel and subjected to Vacuum Liquid Chromatography (VLC) using an isocratic solvent of petroleum ether: ethyl acetate (1:9) and by collecting 20ml fractions to obtain 45 fractions. Based on thin layer chromatographic (TLC) analysis, fraction 6, 7, 8 and 9 were combined to give SB-C which was later subjected to VLC and eluted isocratically using petroleum ether: ethyl acetate (1:9) to obtain eight fractions namely SB-C-1 to SB-C-8 .Fraction 6 showed a prominent single blue spot which was further cleaned using PTLC to obtain **SB-C2.**

**6. Spectra result for compound SB-C2 (Betunilic Acid)**

The Structure of the component was determined spectroscopically using Nuclear Magnetic Resonance Spectroscopy (NMR), 1D-NMR and 2D-NMR, Fourier Transform Infrared Spectroscopy (FTIR) and also by comparing the obtained data with already existing literature. The results obtained are as shown in the tables below.

**IR ($cm^{-1}$):** 2931.42, 1713.93, 1219.58, 933.98, 521.91, 495.23

**$^{1}$H NMR(δ)** 7.5865, 7.5628, 6.8985, 6.8244, 6.2612, 6.2375, 4.7135, 4.5837, 3.9553, 3.9335, 3.6449, 3.1852, 3.1726, 3.1571, 3.1447, 2.9769, 2.3280, 2.3089, 2.2778, 2.2565, 2.2483, 2.2249, 2.2178, 2.2089, 2.1858, 2.1796, 2.1564, 2.1490, 2.0229, 2.0142, 1.9879, 1.9775, 1.9622, 1.9495, 1.9338, 1.9202, 1.6665, 1.6334, 1.6118, 1.5830, 1.5707, 1.4983, 1.4894, 1.4757, 1.4648, 1.4352, 1.4271, 1.4171, 1.3948, 1.3807, 1.3705, 1.3509, 1.2786, 1.2308, 1.1971, 1.1849, 1.1460, 1.1140, 1.0777, 1.0691, 1.0603, 1.0505, 1.0278, 1.0166, 0.9969, 0.9853, 0.9515, 0.9418, 0.9152, 0.8985, 0.8755, 0.8687, 0.8576, 0.8403, 0.8210, 0.8001, 0.7847, 0.7316, 0.6710, 0.6560, 0.6477

**$^{13}$C NMR(δ):** 150.4123, 109.7041, 79.0180, 77.2210, 56.2366, 55.3593, 50.5426, 49.2678, 46.8730, 42.4524, 40.7093, 38.8719, 38.7270, 38.3636, 37.2161, 37.0266, 34.3428, 32.1504, 30.5442, 29.7039, 27.9901, 27.4046, 25.5159, 22.6964, 20.8653, 19.3729, 18.2996, 16.1359, 16.0272, 15.3508, 14.6996, -0.0034, 179.2092

**7. Results and Discussion**

The phytochemical screening of the stem bark of *Sarcocephalus latifolius* revealed the presence of carbohydrate, anthraquinones, cardiac glycoside, saponins, steroid triterpenes, tannins and alkaloids (Table 1)

The IR Spectrum ($cm^{-1}$)of SB-C2 shows main absorption band at 2931.42 $cm^{-1}$ which is typical of OH stretching vibration in an alcohol while another strong absorption observed at 1713.93 $cm^{-1}$ may be due to a carbonyl (C=O) of the carboxylic acid in the molecule. The signal at 521.91 is typical for C=C olefinic. (Figure 1)

The 1H NMR spectrum (δ) of SB-C2 (Fig 2) revealed the presence of lupine type carbon skeleton. The signals at δ4.58 and δ3.93 appear as a proton doublet which indicates an exomethylene group while the signals observed at δ3.16 and δ2.98 could be due to secondary carbinol. Again, the broad spectrum at δ 1.63 is indicative of a vinyl methyl group. The signals at δ 0.66, 0.73, 0.90, 0.94 and 0.99 are due to five tertiary methyl groups. These data are typical of a pentacyclic triterpenoid of the Betunilic acid and comparison with published data suggested it to be Betunilic acid (Robert and Samir (2004)

The $^{13}$C NMR spectrum (Figure 3) showed thirty (30) major recognizable carbon signals. Six methyl groups at δ 27.99 (C-23), 15.35 (C-24), 16.02 (C-25), 16.13 (C-26), 14.69 (C-27), 19.37 (C-30) and an exomethylene groups at 150.41 (C-20), 109.70 (C-29) and a secondary hydroxyl bearing carbon 79.01 (C-3) and a carbonyl group at 179.20 (C-28) (Table 2). In addition, twelve methylene groups, six methyl and six quaternary carbon atoms from the DEPT experiment were observed (Figure 4).The chemical shifts at δ150.41, 179.20 and 109.70 were characteristic peaks for Betunilic type of skeleton, which are due to C-20, C-28 and C-29 respectively (Ahmad, 1994).Other spectra from the 2D NMR really correlated this compound to be a Betunilic acid (Figure 5).

**8. Bioactivity of SB-C2 (Betunilic acid)**

The compound was found to be active on all micro organisms used in the work with the exception of Corynebacterium ulcerans. The zones of inhibition observed for the pathogens range between 21 to 34 mm .From Tables 3 and 4, it was observed that the MIC for the organisms were all 12.5 µg/ml with the exception of *Salmonella*

*typhi* and *Shigellia dysenteric* that had MIC values of 6.25 µg/ml respectively. The MBC/MFC values were recorded for *Staphylococcus aureus, Streptococcus pyrogenes, Bacillus subtilis, Escherichia coli, proteus vulgaris, salmonella typhi, Shigellia dysenteric* and *Candida virusei* were all 25 µg/ml but it was observed for *Methicillin Resistant Staphylococcus Aureus (MRSA), Proteus mirabilis, Pseudomonas aeruginosa, Candida albicans* and *Candida tropicalis* to be 50 µg/ml respectively. From Table 5, SB-C2 has a wider broad spectrum of activities when compared to the standard drug Sparfloxacin used in this study.

**9. Conclusion**

The isolation of Betunilic acid from the plant whose bioactivity was established from this work to be more active as a drug than Sparfloxacin more than justifies why the plant really serves as a general purpose antibiotic in traditional medicine in our society.

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**Table 1: Preliminary phytochemical screening of the stem bark of *Sarcocephalus latifolius***

| Property tested | Stem |
|---|---|
| *Carbohydrate* | + |
| *Glycoside* | _ |
| *Anthraquinones* | + |
| *Cardiac glycoside* | + |
| *Saponins* | + |
| *Steroidal triterpenes* | + |
| *Flavonoids* | _ |
| *Tannins* | + |
| *Alkaloids* | + |

KEYS: + → Present – → Absent

**Table 2: $^{13}$C NMR data assignment for SB-C2 (Betunilic acid)**

| Carbon Position | $^{13}$C Shift ppm [a]Lit. Value | $^{13}$C Shift ppm [b]Lit. Value | $^{13}$C Shift Experimental | $CH_n$ |
|---|---|---|---|---|
| 1 | 38.70 | 39.0 | 38.36 | $CH_2$ |
| 2 | 27.40 | 27.6 | 27.40 | $CH_2$ |
| 3 | 78.90 | 78.2 | 79.01 | CH |
| 4 | 38.80 | 39.1 | 38.87 | C |
| 5 | 55.30 | 55.5 | 55.35 | CH |
| 6 | 18.30 | 18.4 | 18.29 | $CH_2$ |
| 7 | 34.30 | 34.5 | 34.34 | $CH_2$ |
| 8 | 40.70 | 40.8 | 40.70 | C |
| 9 | 50.50 | 50.7 | 50.54 | CH |
| 10 | 37.20 | 37.3 | 37.21 | C |
| 11 | 20.80 | 21.0 | 20.86 | $CH_2$ |
| 12 | 25.50 | 25.7 | 25.51 | $CH_2$ |
| 13 | 38.80 | 38.1 | 38.72 | CH |
| 14 | 42.40 | 42.5 | 42.45 | C |
| 15 | 30.50 | 30.2 | 30.54 | $CH_2$ |
| 16 | 32.10 | 32.9 | 32.15 | $CH_2$ |
| 17 | 56.30 | 47.1 | 56.23 | C |
| 18 | 46.80 | 48.1 | 46.87 | CH |
| 19 | 49.20 | 49.2 | 49.26 | $CH_2$ |
| 20 | 150.30 | 150.1 | 150.41 | C |
| 21 | 29.70 | 30.6 | 29.70 | $CH_2$ |
| 22 | 37.00 | 37.0 | 37.02 | $CH_2$ |
| 23 | 27.90 | 27.9 | 27.99 | $CH_3$ |
| 24 | 15.30 | 15.5 | 15.35 | $CH_3$ |
| 25 | 16.00 | 16.4 | 16.02 | $CH_3$ |
| 26 | 16.10 | 16.7 | 16.13 | $CH_3$ |
| 27 | 14.70 | 15.0 | 14.69 | $CH_3$ |
| 28 | 180.50 | 180.3 | 179.20 | C00H |
| 29 | 109.60 | 108.9 | 109.70 | $CH_2$ |
| 30 | 19.40 | 19.6 | 19.37 | $CH_3$ |

$^{13}$C NMR Litt. Values ([a]Mahato and Kundu 1994, [b] Prince P. Sharma *et al, 2010*)

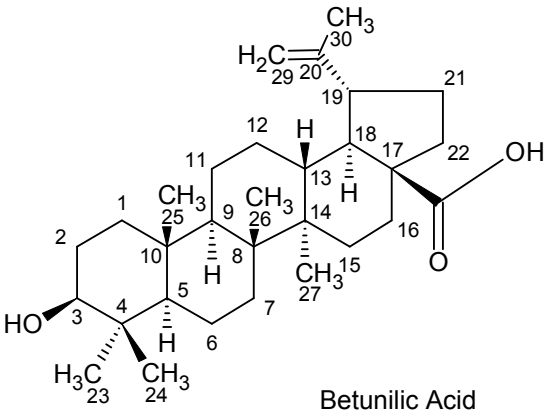


Fig.5

Betunilic Acid

**Table 3: Minimum inhibition Concentration of SB-C2 against the test microbes**

| Test Organism | 50µg/ml | 25.5µg/ml | 12.5µg/ml | 6.25µg/ml | 3.125µg/ml |
|---|---|---|---|---|---|
| *MRSA* | – | – | O* | + | ++ |
| *Staphylococcus aureus* | – | – | O* | + | ++ |
| *Streptococcus Pyrogenes* | – | – | O* | + | ++ |
| *Bacillus Subtilis* | – | – | – | O* | ++ |
| *Corynebacterium Ulcerans* | | | | | |
| *Escherichia Coli* | – | – | O* | + | ++ |
| *Proteus Mirabilis* | – | – | O* | + | ++ |
| *Proteus Vulgaris* | – | – | O* | + | ++ |
| *Pseudomonas aeruginosa* | – | – | O* | + | |
| *Salmonella typhi* | – | – | – | O* | + |
| *Shigellia dysenteric* | – | – | – | O* | + |
| *Candida albicans* | – | – | O* | + | ++ |
| *Candida Virusei* | – | – | O* | + | ++ |
| *Candida Tropicalis* | – | – | O* | + | ++ |

*Key: - =No colony growth, **O*** =MIC, + =light growth, ++ = Moderate colonies growth*

**Table 4: Minimum Bactericidal/Fungicidal Concentration of SB-C2 against the test microbes**

| Test Organism | 50µg/ml | 25.5µg/ml | 12.5µg/ml | 6.25µg/ml | 3.125µg/m |
|---|---|---|---|---|---|
| *MRSA* | **O*** | + | ++ | +++ | ++++ |
| *Staphylococcus aureus* | _ | **O*** | + | ++ | +++ |
| *Streptococcus Pyrogenes* | _ | **O*** | + | ++ | +++ |
| *Bacillus Subtilis* | _ | **O*** | + | ++ | +++ |
| *Corynebacterium Ulcerans* | | | | | |
| *Escherichia Coli* | _ | **O*** | + | ++ | +++ |
| *Proteus Mirabilis* | **O*** | + | ++ | +++ | ++++ |
| *Proteus Vulgaris* | _ | **O*** | + | ++ | +++ |
| *Pseudomonas aeruginosa* | **O*** | + | ++ | +++ | ++++ |
| *Salmonella typhi* | _ | **O*** | + | ++ | +++ |
| *Shigellia dysenteric* | _ | **O*** | + | ++ | +++ |
| *Candida albicans* | **O*** | + | ++ | +++ | ++++ |
| *Candida Virusei* | _ | **O*** | + | ++ | +++ |
| *Candida Tropicalis* | **O*** | + | ++ | +++ | ++++ |

*Key: - =No colony growth, **O*** =MIC, + =light growth, ++ = Moderate colonies growth*

**Table 5: Minimum Inhibition Concentration of Sparfloxacin against the test microbes**

| Test Organism | 50µg/ml | 25.5µg/ml | 12.5µg/ml | 6.25µg/ml | 3.125µg/m |
|---|---|---|---|---|---|
| *MRSA* | – | **O*** | + | ++ | +++ |
| *Staphylococcus aureus* | – | – | **O*** | + | ++ |
| *Streptococcus Pyrogenes* | – | – | **O*** | + | ++ |
| *Bacillus Subtilis* | – | – | **O*** | + | ++ |
| *Corynebacterium ulcerans* | –* | **O*** | + | ++ | +++ |
| *Escherichia Coli* | – | **O*** | + | ++ | +++ |
| *Proteus Mirabilis* | – | **O*** | + | ++ | +++ |
| *Proteus Vulgaris* | – | **O*** | + | ++ | +++ |
| *Pseudomonas aeruginosa* | – | – | – | – | – |
| *Salmonella typhi* | – | **O*** | + | ++ | +++ |
| *Shigellia dysenteric* | – | – | **O*** | + | ++ |
| *Candida albicans* | **Θ** | **Θ** | **Θ** | **Θ** | **Θ** |
| *Candida Virusei* | **Θ** | **Θ** | **Θ** | **Θ** | **Θ** |
| *Candida Tropicalis* | **Θ** | **Θ** | **Θ** | **Θ** | **Θ** |

*Key: **-** =No colony growth, **O*** =MIC, + = scanty colonies growth, ++ = Moderate colonies growth, +++ = Heavy colonies growth*

**FIGURE 1: Infrared spectra of SB-C2 (Betunilic acid)**

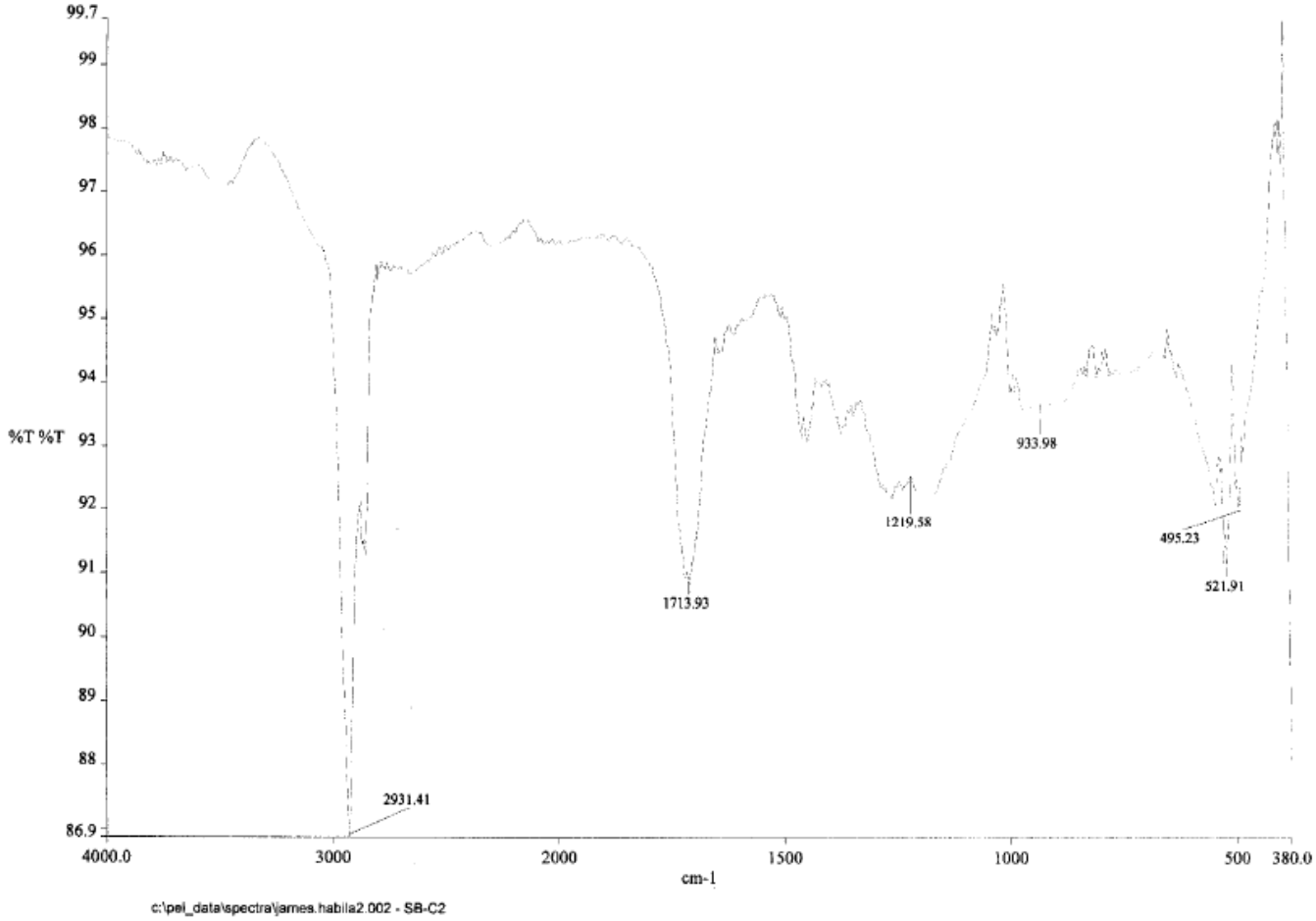

**Figure 2: $^{1}$H NMR spectra of SB-C2**

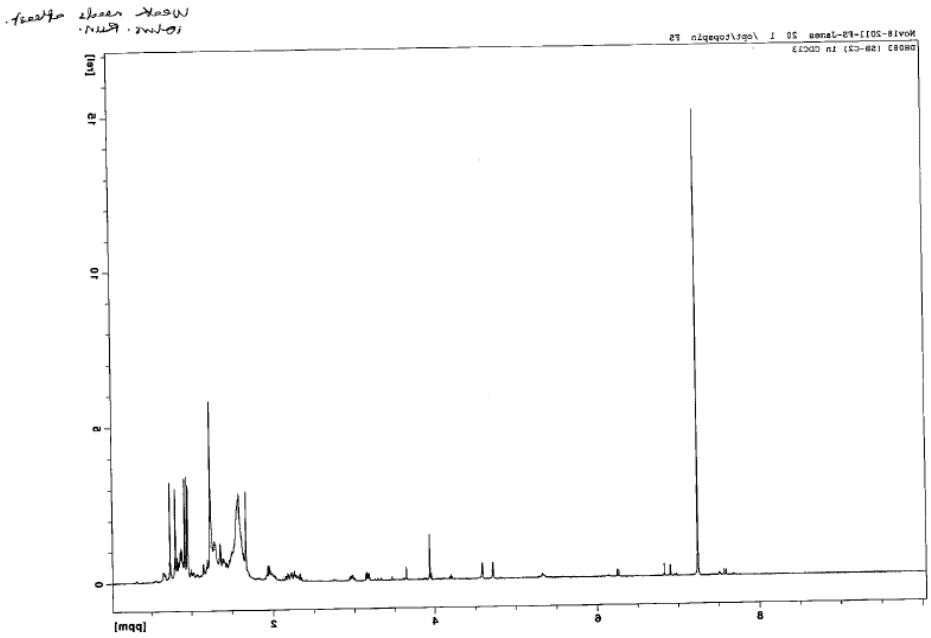

**Figure 3: $^{13}$C NMR spectra of SB-C2**

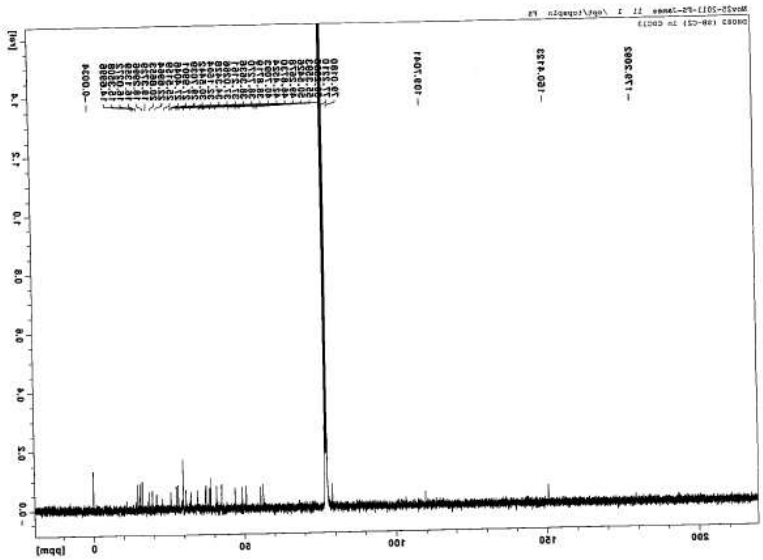

**Figure 4: DEPT-135 spectra of SB-C2**