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# Isolation and Characterization of Bis(2-Ethylhexyl) Benzene-1,2-Dicarboxylate from the Roots of *Detarium microcarpum*

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Bis(2-Ethylhexyl) benzene-1,2-dicarboxylate,
*Detarium microcarpum*,
Chromatography,
Nuclear Magnetic Resonance,
Isolation

## Abstract

The research aimed to Isolate the active constituent in methanolic root extract of *Detarium microcarpum.* The crude root extract of methanolic of *Detarium microcarpum* extracted with methanol by maceration method and concentrated to dryness. The crude methanol extract root of *Detarium microcarpum* yielded 9.65 % ($^{w}/_{w}$) while the partitioned portion yield of n-hexane 18.5 % ($^{w}/_{w}$) Chloroform 24.17 % ($^{w}/_{w}$), Ethyl acetate 5.2 % ($^{w}/_{w}$) and n-butanol 10.7 % ($^{w}/_{w}$) respectively. The phytochemical studies of the crude extract root of *Detarium microcarpum* and partitioned portion of ethyl acetate and n-butanol revealed the presence of carbohydrate, tannins, flavonoid, terpenoids, cardenolides, saponins and cardiac glycoside. The n-butanol partitioned portion of *Detarium microcarpum* was subjected to column chromatographic (CC). Fractions with similar retardation factor ($R_f$) were combined, coded and subsequently screened for phytochemicals. Fractions AIZ (B) gave the structure which was established using spectroscopic technique and characterized as Bis(2-Ethylhexyl) benzene-1,2-dicarboxylate.

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## Introduction

The use of medicinal plants in the treatment of diseases has generated renewed interest in recent times, as herbal preparations are increasingly being used in human healthcare systems. Medicinal plants play a golden role not only as traditional medicine but also as trade commodities, meeting the demand of distant markets for the development of new drugs. In fact, to realize the effective integration of plants into a medical system, researchers and practitioners should be trained in both modern and traditional medicine in the use of plant compounds. In addition, to build credibility for the use of plants in conventional medicine, the empirical arguments should be converted into evidence-based arguments [1]. Medicinal plants are used for treatment because they have certain

properties, including synergistic actions. The constituents of the plant may interact with each other and this interaction can be beneficial for both or adverse to either of them or eliminate the harmful effects of both. Plant-derived compounds can dramatically improve hard-to-treat illnesses such as cancer. Plant components are also characterized by their ability to prevent the development of certain diseases. The toxicity and adverse effects of conventional and allopathic medicines have also been important factors in the sudden increase in population demands and increase in the number of herbal drug manufactures as well as a reduction in the use of chemical drugs [2]. Traditional medicine has been used for thousands of years with great contributions made by practitioners to human health, particularly as primary health care providers at the community level and has maintained its popularity worldwide. About 60-85% of the population in every country of the developing world has to rely on traditional medicine. The practice of traditional Medicine is widespread in China, India, Japan, Pakistan, Sri Lanka, Thailand and Korea [3]. Medicinal herbs have a hopeful future since there are about half a million plants around the world, most of them have not yet been studied in medical practice and current and future studies on medical activities can be effective in treating diseases [4]. The beginning of the development of herbal medicines was concurrent with the development of chemistry and isolation, purification and determination of plant compounds. In the past, drug discovery of the biological compounds from plant materials and the process of identifying the structures of active compounds from the extracts were problematic depending on the complexity of the compounds and might take weeks, months or even years. Nowadays, the rate of bioassay-guided fractionation has been significantly enhanced by the development of precision instruments such as high-performance liquid chromatography-Mass spectrometry (HPLC/MS), liquid chromatography-mass spectrometry (LC/MS), magnetic field and nuclear magnetic resonance is a recent major breakthrough for the categorization of compounds that are extremely limited in quantity in their organisms of origin [5].

## Materials and Methods

Different equipment/instruments have been used during the course of this research, which include: Fourier Transform Infrared perkin-Elmer Spectrum Express Version 1.20.200, Nuclear Magnetic Resonance Bruker Topspin 3.2 Spectrum at 500MHZ, other apparatus such as polyethylene Bags wooden motar and piston, beakers, separating funnels, glass funnel, stirring rod, conical flasks, spatula, steel trays, test tubes, measuring cylinders, Whatman No. 1 filter papers, muslin cloth, columns glass tube (90×2.8cm), capillary tubes, chroma tanks, retort stands, wash bottles, desiccators, Bunsen burner, cotton wool among others.

**Chemicals and Reagents/Solvent**

All reagents used were of analar grade (Riedel-de-Haen, Merck, Germany, JHD china and Sigma Aldrich, Germany). The silica gel for the column chromatography was of mesh size (60-120 G Merck, Germany and thin layer chromatography (Kieselgel 60 G Merck) made to a thickness of 0.25 mm, recoated aluminum TLC plates of 20 × 20 mm.

**Sample Collection and Identification**

Fresh sample of the root of *Detarium microcarpum* was collected from Sangere in Girei Local government Adamawa State, Nigeria and authenticated by Plant Taxonomist with voucher number UMC/DC/343. The samples were dried under shade, rendered free of foreign materials through manual picking and grounded with a wooden mortar and pestle to fine powders.

**Plant Extraction**

Three kilograms (3 kg) of the pulverized dried root of *Detarium microcarpum* were macerated with absolute methanol at room temperature for seventy-two hours (72hour) in a round bottom flask with occasional shaking. The soaked sample were passed through a muslin cloth to remove the vegetative debris and the liquids were then filtered through Whatman No, 1 filter paper. The crude extracts were concentrated to dryness at room temperature. The extracts were weighed, labeled, and subjected to further analysis. 200g of the crude extracts were subjected to defatting with n-hexane and then partitioning with chloroform, ethyl acetate, and n-butanol based on increasing order of polarities using the dry partitioning technique.

**Column Chromatography (CC)**

A glass tube with a diameter of 2.8 cm and a height of 90 cm with a tap at the bottom was used for the column chromatographic technique. A plug of cotton wool was well placed at the bottom of the column very close to the tap so as to prevent the stationary phase from blocking the column. Two hundred and fifty of silica gel 60-120 mesh (Loba Chemie, India) was used to prepare by dry method. The silica gel was put into the column packed cautiously and manually to about two third the size of the column tube using a glass funnel. The chloroform was then added to the silica gel inside the column continuously for proper packing until it became homogenous. The gel was then allowed to settle and pack for 24 hrs. The air bubbles were avoided and care was taken not to dry the column by maintaining the level of the chloroform to that of the silica gel. The sample (15 g) of the mixture of the n-butanol partitioned portion and 5 g of silica gel in methanol was mounted on the already equilibrated silica-fixed column on top of the stationary phase. This was topped with a small layer of cotton, then sand to protect the

shape of the organic layer from the velocity of the newly added eluent (stationary phase). The eluting solvent initially was 100 % chloroform and the polarity were gradually increased by 80:20, chloroform: methanol ratio until 0:100 chloroform: methanol ratio was used.

**Thin Layer Chromatography (TLC)**

Commercially prepared aluminum back TLC plates of 20 x 20 cm x 0.25 mm activated silica gel of 60 $F_{254}$ (Merck, Germany) were cut to the size of 5 x 5 cm. n-butanol partition portion of *Detarium microcarpum* which contained the most phytochemicals and had the largest quantity was preferred for the chromatographic analysis. Methanol was used to dissolve the partition portion and allowed to stand for 30 minutes and then it was spotted at the bottom of the TLC plate (about 2 cm from the base). The partitioned portion which was dissolved in a few drops of methanol was dried in air for 30 minutes and was used for the spotting with the aid of a capillary tube. The spotted plate was kept in a previously saturated developing chamber containing the chosen solvent system as the mobile phase and was covered with a watch glass and allowed to run 3/4$^{th}$ of the height of the prepared plates [6].

A solvent combination of ethyl acetate, formic acid, acetic acid and water was used in the ratio combination of 100:11:11:26 respectively. The fractions collected were monitored by TLC and similar fractions were pooled together. The solvent was then evaporated from the bulked fraction, allowed to dry and weighed. TLC analysis was carried out on the semi-dry bulked fraction using various polar solvents to ensure the purity of the fraction. The fraction which gave a single spot when developed using the chromatographic tank, sprayed with 10 % sulphuric acid in methanol was allowed to dry and viewed under ultra-violet light of 254 nm wavelength. A crystalline solid was obtained which was soluble in methanol and ethyl acetate.

**Infrared (IR) Spectroscopy**

The infrared spectrum of the compounds was measured using Fourier Transform Infrared (FTIR) spectrometer according to the manufacturer's protocol. The IR spectra were identified and analyzed by the spectra software provided in the spectrometer system.

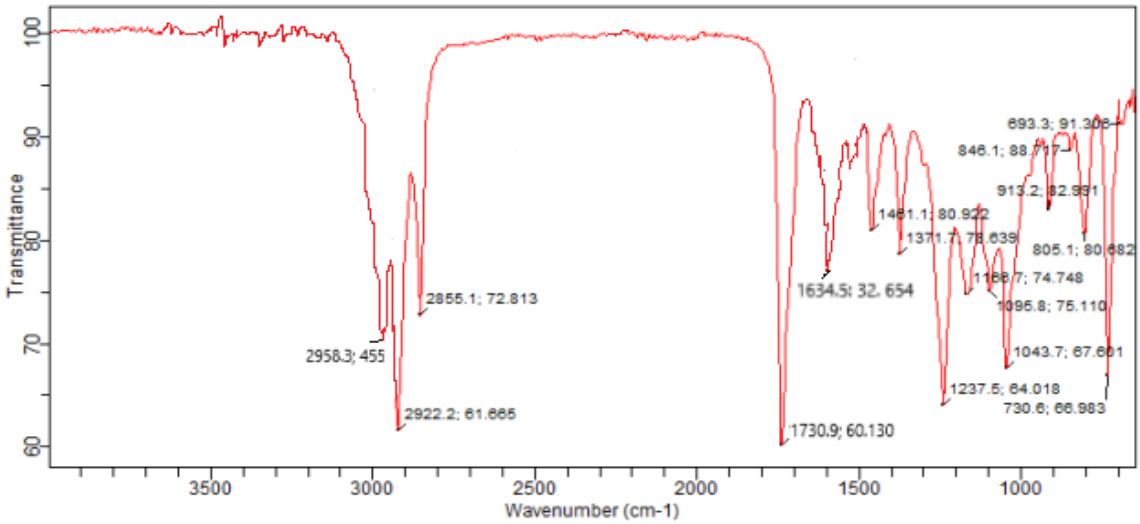


**Figure 1: Fourier Transform Infrared Spectra of Compound AIZ**

**Nuclear Magnetic Resonance (NMR) Spectrometry**

NMR experiments were performed using an NMR spectrometer in Ahmadu Bello University Zaria (A.B.U) NMR laboratory. Sample dissolved in deuterated Dimethylsuphoxide (DMSO-d6) and filtered through cotton wool pugged Pasteur pipette and then placed in NMR tubes. A very small amount of tetramethylsilane (TMS) was added to the sample solution to be used to reference the 1H and 13C spectra. All resonance assigned was related to TMS. The NMR experiments were analyzed using "TopSpin" software under automation controlled by "icon nor". The 1D, 1H, and 13C NMR experiments were carried out. In addition, homo and heteronuclear 2D experiments, correlated Spectroscopy with gradients (g-COSY) and Heteronuclear Multiple-Quantum Correlation (HMQC) spectra were also acquired. All pulse sequences used were of the instrument's standard.

**Thin Layer Chromatographic Analysis of Column Fraction of n-butanol Root Partition Portion of *Detarium microcarpum***

The fractions collected from column chromatographic analysis were monitored by using the TLC technique. The combined column fractions afforded subfraction encoded AIZ sub fraction show a good resolution on TLC plate solvent system of ethyl acetate, formic acid, acetic acid and water in the ratio of 100:11:11:26. was

used. AIZ is brown in color and given a spot. The $R_f$ value of the spot were 0.63.

## Results

### Infrared Spectroscopy

The IR spectra of compound AIZ (Fig 1 and Table 1) showed a broad-spectrum band at 2958.3 $cm^{-1}$indicating the presence of an aromatic compound. The 2922.2 $cm^{-1}$corresponds to absorption by -$CH_3$ (alkyl residue) while a sharp band at 2855.1$cm^{-1}$ corresponds to CH in $CH_2$. A signal at 1730.9 $cm^{-1}$ indicate C=O (cyclic ketone), 1634.5 $cm^{-1}$ for C=C aromatic stretching vibration and at 1237.5 $cm^{-1}$ absorption band for -O- group.

**Table 1: Summary of IR Spectra of Compound AIZ**

| Wave number | Functional group |
|---|---|
| 2958.3 | =C-H aromatic |
| 2922.2 | C-H in $CH_3$ |
| 2855.1 | C-H in $CH_2$ |
| 1730.9 | C=O |
| 1634.5 | C=C |
| 1237.5 | -O- |

### $^1$HNMR

The $^1$HNMR spectrum (Fig 2 and Table 2) showed a doublet at 8.03 ppm (d, 2H doublet) δ 7.72 (d, Triplet) δ 4.66 (d, doublet), δ 1.89 (d, Pentent), δ 1.19 (q, quartet), δ 1.25 (pentent), δ 1.31 (sextet), δ 0.88 (triplet), δ 1.55 (quartet), δ 0.99 (triplet).

**Table 2: Summary of the $^1$HNMR of Compound AIZ**

| Position | Chemical shift Values expt | Chemical shift Values Calc | Multiplicities |
|---|---|---|---|
| 5,8 | 8.06 | 8.03 | Doublet |
| 6,7 | 7.86 | 7.72 | Triplet |
| 9,17 | 4.56 | 4.66 | Doublet |
| 10,18 | 1.69 | 1.89 | Pentet |
| 11,19 | 1.14 | 1.19 | Quartet |
| 12,20 | 1.26 | 1.25 | Pentet |
| 13,21 | 1.30 | 1.31 | Sextet |
| 14,22 | 0,89 | 0,88 | Triplet |
| 15,23 | 1.66 | 1.55 | Quartet |
| 16,24 | 1.02 | 0.99 | Triplet |

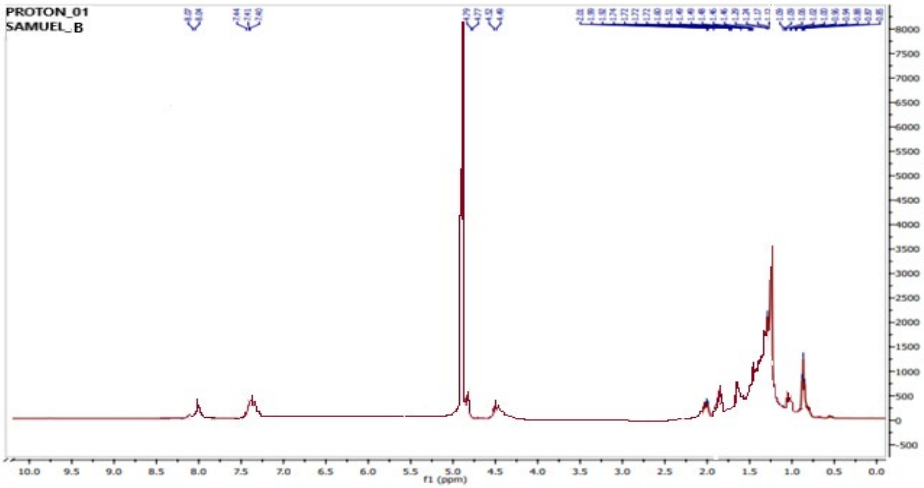


**Figure 2: $^1$HNMR Spectra of Compound AIZ in $d_6$-DMSO at 500 MHZ**

### $^{13}$CNMR

The $^{13}$CNMR spectrum (Fig 3) showed the presence of 24 carbon atoms. The chemical shift signal at δ 67.6, 132.9, 132.4, 167.8 is an indicative of quartinary carbon C, and δ 128.0, 132.8, 132.7, 128.0, 39.6, are carbon to hydrogen CH, while signal at δ 66.9, 30.8, 29.3, 23.0, 23.7, corresponds to $CH_2$ and δ 14.1, 11.6, are indicative of Methyl $CH_3$. (Figure 4). The Dept signal revealed a total of 4 quartinary carbon atoms at $C_1$, $C_2$, $C_3$, and C4 respectively while six methine were observed at $C_5$, $C_6$, $C_7$, $C_8$, $C_{10}$ and $C_{18}$. A total of ten methylene ($CH_2$) were also revealed at $C_9$, $C_{11}$, $C_{12}$, $C_{13}$, $C_{15}$, $C_{17}$, $C_{19}$, $C_{20}$, $C_{21}$ and $C_{23}$. And a total of four methyl ($CH_3$) were observed at $C_{14}$, $C_{16}$, $C_{22}$ and $C_{24}$ (Table 3).

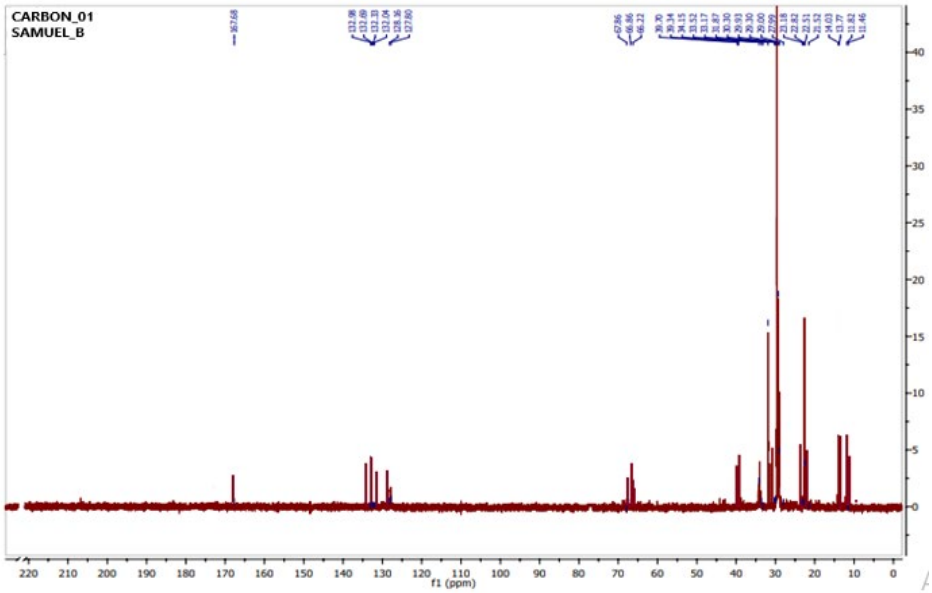


Figure 3: $^{13}$CNMR Spectra Compound of AIZ in $d_6$-DMSO at 500 MHZ

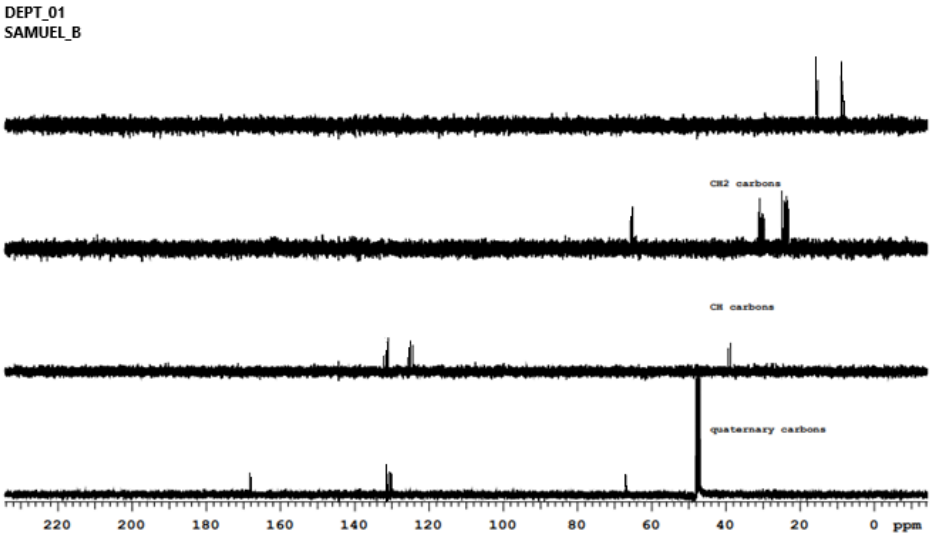


Figure 4: DEPT 90 spectra of Compound AIZ in $d_6$-DMSO at 500 MHZ

Table 3: Summary of the $^{13}$CNMR Compoound AIZ

| Position | Chemical shift expt | Chemical shift calc | DEPT |
|---|---|---|---|
| 1 | 66.8 | 67.6 | C |
| 2 | 132.6 | 132.9 | C |
| 3 | 132.2 | 132.4 | C |
| 4 | 167.9 | 167.8 | C |
| 5 | 128.6 | 128.0 | CH |
| 6 | 133.2 | 132.8 | CH |
| 7 | 132.4 | 132.7 | CH |
| 8 | 128.6 | 128.0 | CH |
| 9 | 66.5 | 66.9 | $CH_2$ |
| 10 | 38.6 | 39.6 | CH |
| 11 | 30.6 | 30.8 | $CH_2$ |
| 12 | 29.6 | 29.3 | $CH_2$ |
| 13 | 23.0 | 23.0 | $CH_2$ |
| 14 | 14.4 | 14.1 | $CH_3$ |
| 15 | 23.8 | 23.7 | $CH_2$ |

| | | | |
|---|---|---|---|
| 16 | 11.8 | 11.8 | $CH_3$ |
| 17 | 66.5 | 66.9 | $CH_2$ |
| 18 | 38.6 | 39.6 | CH |
| 19 | 30.6 | 30.8 | $CH_2$ |
| 20 | 29.6 | 29.3 | $CH_2$ |
| 21 | 23.0 | 23.0 | $CH_2$ |
| 22 | 14.4 | 14.1 | $CH_3$ |
| 23 | 23.8 | 23.7 | $CH_2$ |
| 24 | 11.8 | 11.6 | $CH_3$ |

Based on the spectral data obtained, the compound (AIZ) is characterized as Bis (2-ethylhexyl) benzene-1,2-dicarboxylate. (Figure 5) with the molecular formula $C_{24}H_{38}O_4$.

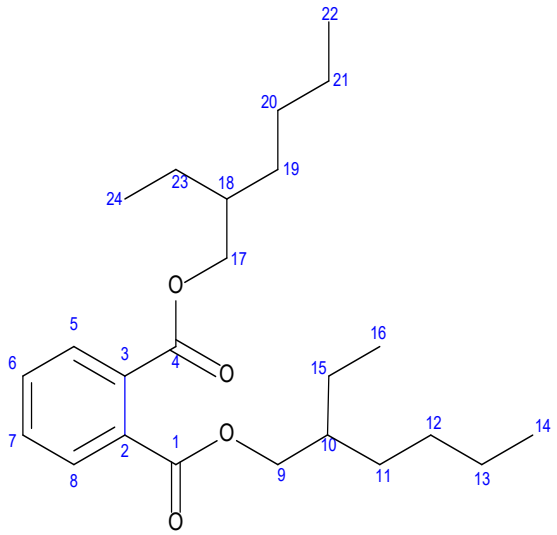


**Figure 5: Compound AIZ Bis(2-ethylhexyl) benzene-1,2-dicarboxylate**

## Discussion

Chromatographic techniques (CC and TLC) showed significant effect in the purification process for the isolation of possible bioactive compounds. The successful separation of biomolecules by chromatographic technique was depended upon suitable solvent system which needs an ideal range of partition coefficient for each target compound. The TLC profiling of the extracts gave an impressive result that directing towards the presence of number of phytochemicals which is present such as carbohydrate, cardenolide, flavonoids, tannins, terpenoids, saponins and glycosides. The isolated compound of fraction AIZ shows a mid Rf value in polar solvent system which indicated the compounds are polar. The mixture of solvents with variable polarity in different ratio can be used for separation of pure compounds from plants extract. The selection of appropriate solvent system for a particular plant extract was achieved by analyzing the $R_f$ values of compounds in different solvent system. In the present state of affairs, TLC profile of the *Detarium microcarpum* in different solvent system indicated the presence of diverse type of phytochemicals in this plant. Different $R_f$ value of the compound also reflects an idea about their polarity.

The structure of compound AIZ was elucidated and characterized using physical and spectroscopic techniques. The IR spectra of compound AIZ showed a broad-spectrum band at 2958.3 $cm^{-1}$ indicate the presence of an aromatic compound. The 2922.2 $cm^{-1}$ corresponds to absorption by $-CH_3$ (alkyl residue) a band at 2855.1$cm^{-1}$ CH in $CH_2$ (saturated alkyl), 1730.9 $cm^{-1}$ for C=O cyclic ketone, 1634.5 $cm^{-1}$ for C=C aromatic stretching vibration and at 1237.5 $cm^{-1}$ absorption band of -O- group.

The $^1$HNMR spectrum showed a doublet at 8.03 ppm (d, 2H doublet) δ 7.72 (d, Triplet) δ 4.66 (d, doublet), δ 1.89 (d, Pentent), δ 1.19 (q, quartet), δ 1.25 (pentent), δ 1.31 (sextet), δ 0.88 (triplet), δ 1.55 (quartet), δ 0.99 (triplet).

The $^{13}$CNMR spectrum showed the presence of 24 carbon atoms. The signal at δ 67.6, 132.9, 132.4, 167.8 indicative of 4 carbon C, δ 128.0, 132.8, 132.7, 128.0, 39.6, are 6 methylidene CH, δ 66.9, 30.8, 29.3, 23.0, 23.7, are 10 methylene $CH_2$ and δ 14.1, 11.6, are indicative of 4 Methyl $CH_3$. This finding is the same as the finding of Swati et al. [7]

The $^{13}$CNMR spectra indicated that the compound had 12 C atoms (Primary, Tertiary, and Secondary) in different electronic environments that are present in aromatic rings, acids, and esters. In depth analysis using $^{13}$C indicated that CH and $CH_2$ molecules were present above 6 C atoms and $CH_2$ molecules were located on top of the remaining 6 C atoms. These findings are with the agreement of Swati et al. [7].

1,2-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester has a molecular formular of $C_{24}H_{38}O_4$ the molecular had one aromatic ring or three pi bonds. Although this compound is well known and has been reported in the literature, it has not been reported as a constituent of *Detarium microcarpum.* This compound was first isolated from *Piper longum* [8], leaves of *Gorgonian melithaea* spp from the south china sea [9], leaf and bark extracts of *salix subserrata* [10]. The compound was comfirmed as phenolic dimer [7].

Bis(2-ethylhexyl) benzene-1,2-dicarboxylate showed a better broad spectrum of antibacterial activity against both gram positive (*Staphylococcus aureus*, *Bacillus subtilis*, and *Sarcina lutea*) and gram negative (*Escherchia coli, Shigella sonnei, Shigella shiga*, and

*Shigella dysenteriae*. The major bacteria that cause diarrhoea and dysentery are *Shigella dysenteriae* and *Escherichia coli.* Bis(2-Ethylhexyl) benzene-1,2-dicarboxylate has been effective in treating these bacteria base on the literature this finding shows that, the probable compound found in *Detarium microcarpum* has been effective in treating diarrhoea.

## Conclusion

The Compound Bis(2-ethylhexyl) benzene-1,2-dicarboxylate or 1,2-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester phenolic dimer can be considered a novel candidate for bioprospection and drug development in the treatment of diarrhoea.

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