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*Full Length Research Paper*

# Didecanoate compound: Isolated from *Momordica charantia* Linn. seeds from Nigeria

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**The seeds of *Momordica charantia* were extracted with hexane, ethyl acetate and 95% ethanol successively by percolation and concentrated at 37°C. Fractions 19-21 and 247 gave two compounds. However, only one has been elucidated to be 4a-phorbol-12, 13-didecanoate using the state- of –art-tools of spectrometry. The phytochemical screening of seeds of *M. charantia* showed the presence of flavonoids, glycosides, sterols, fat and oil in hexane, ethyl acetate and ethanol extracts. Anthraquinone is only present in hexane and ethyl acetate while alkaloid is in hexane and ethanol. And also phyto-medicine should be integrated into the health system of Nigeria and developed in such a way to bring harmony between the traditional and modern system of health care with minimum threat to each other.**

**Key words:** *Momordica charantia,* Cucurbitaceae, gas chromatography, thin layer chromatography, phytochemicals.

## INTRODUCTION

*Momordica charantia* Linn. (bitter melon) belongs to the family of Cucurbitaceae, a climbing vine which is commonly seen growing on walls and shrubs in the tropics. The textured leaves look as a bite has been taken from them giving the plant its Latin name Momordica which means to bite. The orange fruits are soft when ripe and inside black seeds have a red covering. It is used as food, bitter flavouring and medicine (Basch et al; 2003). Earlier claims showed that its bitter fruits have carminative, aphrodisiac, anthelmintic properties and are used in syphilis, rheumatism, and troubles of spleen and ophthalmic. It is also useful in piles, leprosy and jaundice and also used as a vermifuge (Kirtikar and Basu 2006). The ethanolic extract showed an analgesic and antipyretic effect which was significant higher than that in the control rats. The observed pharmacological activities provide the scientific basis to support traditional claims as well as explore some new and promising leads (Roshan et al., 2010). The mineral and amino acid analysis showed that the bitter melon contained nutritionally useful quantities of most of the essential minerals and amino acids. The predominant fatty acid was α- eleostearic acid in non-polar lipids, linolenic acid in glycolipids and palmitic acid in phospholipids (Kuri et al., 1991). The plant inhibits primary human adipocyte differentiation by modulating adipogenic genes and it was effective in reducing lipid accumulating in primary human adipocytes by regulating adipogenic transcription factors and adipocytokine gene expression (Pratibha et al., 2010). Many pharmacological properties have been reported including antioxidant, anti-diabetic, anticancer, anti-fertility, antimicrobial, antiviral and hepatoprotective activity. Popularity of the plant in various systems of traditional medium for several

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ailments such as anti-diabetic, contraceptive, dysmenorrhoea, eczema has been reported. Traditionally, it has also been used in treating peptic ulcers, interestingly in a recent experimental studies have exhibited its potential against *Helicobacter pylori* (Grover, 2004). The plant contains several biologically active compounds chiefly momordicin, cucurbitacin and glycosides such as momordin, charantin, momordicosides and other terpenoids compounds such as momordenol, momordol, momordicin-28 and momordicilin (Fatope et al., 1990; Ortigao and Better, 1792). However, there are reports to our knowledge on isolation of some bioactive compounds from the leaves of *M. charantia* which are Asian variety, hence the present study was undertaken to carry out phytochemicals and to isolate newly identified compounds from the seeds of the bitter melon for the first time with African specie using the state-of -art –tools spectrometry.

## MATERIALS AND METHODS

### Plant materials

The matured gourds of *M. charantia* were harvested at Chika, along Airport Road, Abuja and identified at the Herbarium Unit of Pharmaceutical Research and Development, Idu -Abuja, where voucher specimens were deposited.

### Extraction/ partitioning procedure

Blended 500 g of the seeds was extracted by percolation as described by (Singh et al., 2006) using hexane, ethyl acetate and 95% ethanol as extracting solvents which were in increasing order of polarity and they were concentrated using rotary evaporator at 37°C, at reduced pressure. Thereafter, phytochemical screening was carried out and ethyl acetate extract was subjected to flash column chromatography based on the results of the screening. Using Chemo-glass 500 ml of column diameter 27 mm and optimal flow range of 10-30 ml/min, 10 g Silica gel powder of 150 angstrom spore, 35-75 micron particle and 239-400 mesh size and 5 g of the crude extract dissolved in 15 ml ethyl acetate. The gradient system, in which the solvent composition changes during the course of elution, was used. The solvent system consisted of hexane (Hex), ethyl acetate (Ea), methanol (Meth) in different ratios. 287 fractions of 40 mls were collected and evaporated to dryness. Thin layer chromatography was also used for fingerprint profiling of the fractions collected using solvent mixture of hexane and ethyl acetate (9:2). Those with the same retention values (Rf.) were combined and two major fractions namely samples 19-21 which is denoted as compound PS1 and sample 247denoted as compound PS2 were obtained.

### Apparatus, reagents and equipment

Solvents used were of analytical grade manufactured by Sigma-Aldrich Laboratory, Germany but were redistilled for certainty of quality. Thin layer chromatography (TLC) analysis was carried out on pre-coated Merck-Kiesegel 60F 254 with 0.25 mm thickness. The TLC plates were visualized by exposure to iodine vapor and further clarified by UV light at 254/336 nm. The GC-MS analysis was also carried out. All these were done at the Sheda Science and Technology Complex, Abuja.

### Phytochemical screening of extract

For the purpose of this study, phytochemical screenings were carried out on the extracts to confirm the presence or absence of the following plant secondary metabolites: alkaloids, phenols, sterols, terpenes, tannins, flavonoids, anthraquinones, cardiac glycosides, saponins, fats and oil (Harborne, 1973; Trease and Evans, 1989).

Phenols: Equal volumes of each extract and ferric chloride solution are added together. A deep bluish green precipitate indicates the presence of phenol.

Alkaloids: Each extract was added to 1% aqueous HCl over water bath and filtered. The filtrate was treated with iodine in potassium iodide. Formation brown or reddish brown precipitate indicates presence of alkaloids.

Steroids: Each extract was added to 2 ml acetic anhydride and 2 ml $H_2SO_4$. Colour change from violet to blue or green indicates the presence of steroids.

Terpenes: Each extract was added to 0.5 ml acetic anhydride and few drops of concentrated $H_2SO_4$. A bluish green precipitate indicates the presence of terpenes.

Cardiac glycosides: Extract was treated with 2 ml glacial acetic acid with a drop of ferric chloride solution and underplayed with 1 ml $H_2SO_4$. A browning at the interface indicates the presence of cardiac glycosides.

Tannins: Each extract was boiled in 20 ml water and filtered. A few drops of 0.1% ferric chloride solution were added. Brownish green or blue-black color indicates the presence of tannins.

Flavonoids: 5 ml ammonium solution was added to aqueous filtrate of each extract and then few drops of concentrated $H_2SO_4$. Yellow coloration indicates the presence of flavonoids.

Anthraquinones: 10 ml benzene was added to each extract and filtered. 0.5 ml of 1% Ammonium solution was added and shaken. Pink, red, or violet color in the ammoniacal lower phase indicates the presence of Anthraquinones.

Saponins: 1 g each extract was boiled with 5 ml distilled water and filtered. 3 ml distilled water was added to the filtrate and shaken vigorously for 5 min. Persistent frothing on warming indicates the presence of Saponins.

Fats and Oils: Small quantity of each extract was pressed between two filter papers. Oily stains indicate the presence of fats and oils.

### Spectral analysis

The fraction with higher yield-Compound PS1 was used for this work. It was elucidated and characterised by H-NMR, GC-MS and FTIR and discussed. The other compound PS2 was only analysed by H-NMR, GC-MS and FTIR and Mass Spectrometer search gave a complex compound and was not discussed in this report. Quantitative and qualitative data were determined by GC-MS respectively. The samples were injected into a thermo-scientific trace GC ultra system coupled to DSQ II mass spectrometer, and equipped with an AS 3000 auto sampler and a split/split-less injector. The column used was an TR-5MS, 30 m × 0.25 mm i. d., 0.25 µm d. f., coated with 5% diphenyl-95% polydimethyl siloxane, operated with the following oven temperature programmed: 140°C, held for 1 min, rising at 8°C/min to 300°C, injection temperature and volume, 250° C and 1.0 µl respectively; injection mode, split, split ratio, 15:1; carrier gas, helium at 30 cm/s linear velocity and inlet pressure 99.8KPa; detector temperature, 280°C. The components of the sample were identified based on the basis of their retention indices. Identification confirmation was by comparison of their mass spectra with published spectra (Adams,

**Table 1.** Result for phytochemical screening of *M. charantia* seed extracts

| Phytochemical | Result | | |
|---|---|---|---|
| | Hexane | Ethyl Acetate | 95% Ethanol |
| Alkaloids | + | - | + |
| Flavonoids | + | + | + |
| Glycosides | + | + | + |
| Saponins | - | - | + |
| Taninns | - | + | - |
| Sterols | + | + | + |
| *Terpenes* | + | + | - |
| Phenols | - | - | - |
| Anthraquinones | + | + | - |
| Fats and Oils | + | + | + |

Key: (+) = Present; (-) = Absent.

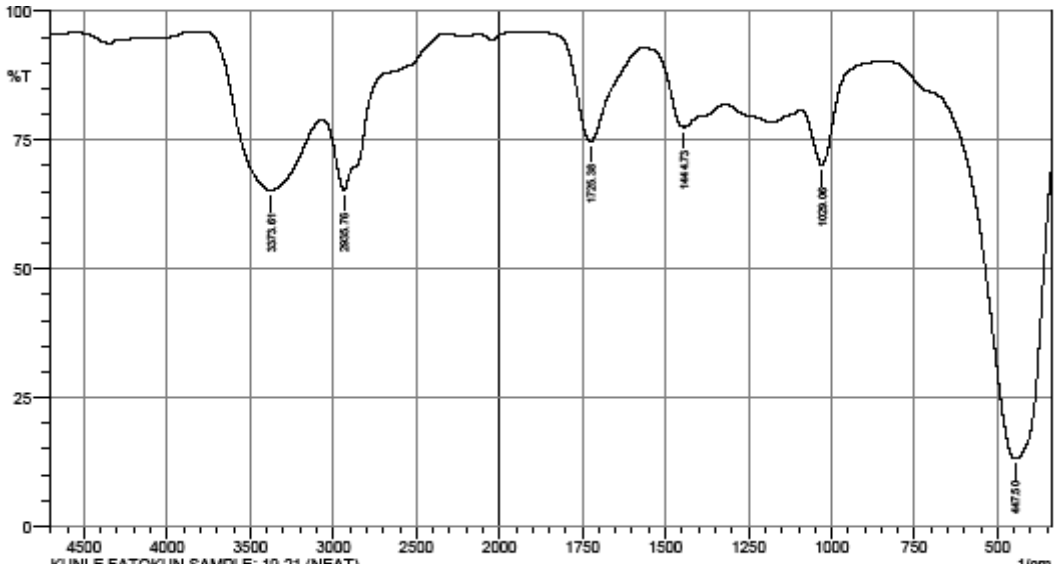


**Figure 1.** Infra red spectrum of compound PS1.

1989) and those of reference compounds from Library of National Institute of Standard and Technology (NIST) database.

## RESULTS AND DISCUSSION

The phytochemical screening of seeds of *M. charantia* showed the presence of flavonoids, glycosides, sterols, fat and oil in hexane, ethyl acetate and ethanol extracts in Table 1. Only hexane and ethanol extracts contain alkaloids while phenols were not detected in all the extracts. These chemical constituents present in the extracts have many therapeutic values. Flavonoids have both antifungal and antibacterial activity. They possess anti-inflammatory properties (Iwu et al., 1999). GC-MS analysis and FTIR, HNMR Spectral analyses of Compound PS1 gave the following results. From Figure 1 of the FTIR analysis, the intense and broad absorption band at 3373.61 $cm^{-1}$ is associated with both the O-H and the C-O stretching vibrations; 1444.73 $cm^{-1}$ is suggestive of C–C stretch (in-ring); 1725.38 $cm^{-1}$ is suggestive of esters. This is confirmed by the C–O stretches which appear as two or more bands in the region 1029.06 $cm^{-1}$ typical of esters. The band at 2935.76 $cm^{-1}$ is characteristic of spectrum of simple alkanes due to C–H stretching and bending. This is confirmed by the C–H bend or scissoring of 1444.73 $cm^{-1}$ for alkanes. Thus from these results the compound is an alcoholic ester with extended and saturated alkyl chains. From Figure 5 of the H-NMR analysis, it showed chemical shifts at 0.86 ppm suggestive of terminal methyl -$CH_3$ in alkyl; 1.2-1.49 ppm

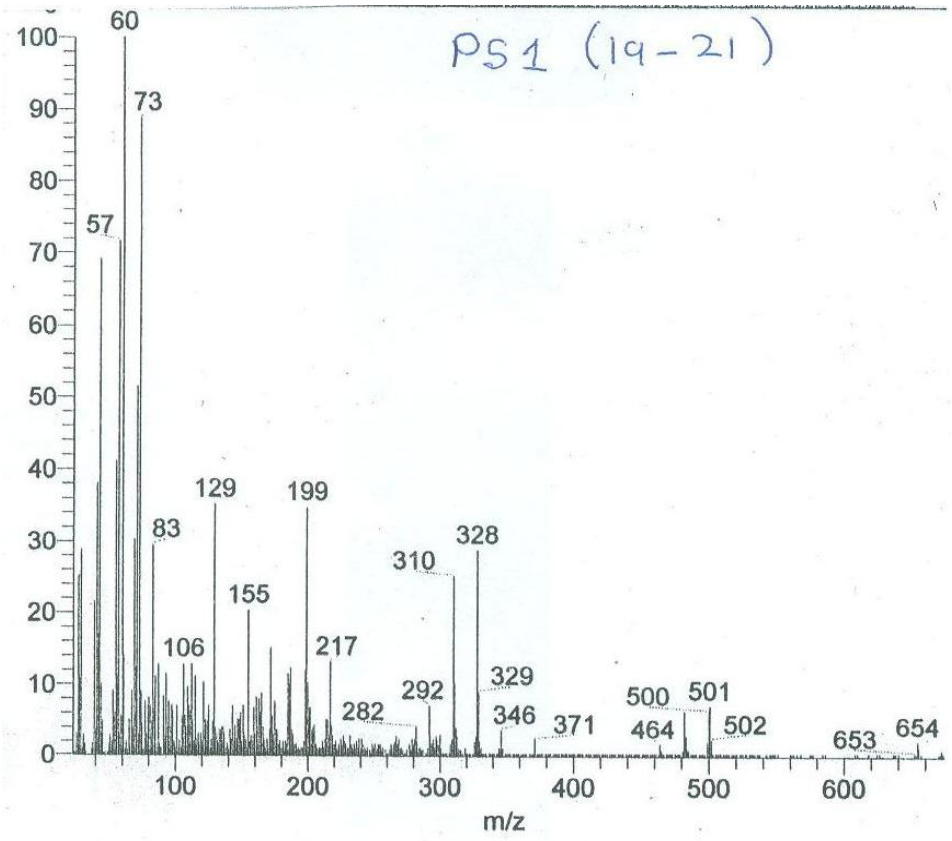


**Figure 2.** Mass spectrum of compound PS1.

is suggestive of saturated alkyl chain -$CH_2$; 3.5 ppm is suggestive of hydroxyl proton R-OH; 4.25-4.4 ppm is suggestive of esterified glycerol -$CH_2$-O-CO-R or esterified phorbol. From Figure 2 of the GC-MS, the gas chromatography showed one major peak at with retention time of 2.95 min. Thus it has one component.

The mass spectral showed a molecular ion at m/e 654 with a base peak at m/e 60. Other major peaks include 501, 328, 310, 199, 129, 83 and 73. From the computer MS Library in Figure 3, phytochemical is suggestive of 4a-Phorbol, 12, 13-didecanoate and its fragmentation pattern was showed in Figure 4. Also, from Figure 5 of the H-NMR analysis, it showed chemical shifts at 0.86ppm suggestive of terminal methyl —CH3 in alkyl; 1.2-1.49ppm is suggestive of saturated alkyl chain -CH2; 3.5ppm is suggestive of hydroxyl proton R—OH; 4.25-4.4ppm is suggestive of esterified glycerol —CH2—O—CO—R or esterified phorbol. From the ethno medical uses and biological studies the seeds were reported to be anti diabetic. This agrees with report that showed that decanoic acid found attached to phorbol as obtained above had the same modulating effect on the sub-family of nuclear receptors called Perixisome Ploriferator Activited receptors (PPAR) as the anti-diabetic drug-Thiazolidinedione (TDZ). These receptors are responsible for glucose metabolism. This may be responsible for its reported anti-diabetic therapeutic properties. They concluded that this acid can serve as a regulator of blood sugar levels in cells and may have important application in designing better and safer drugs for diabetes treatment since TDZ has negative side effects which include weight gain, fluid retention and increased risk for cardiovascular disease. Based on the foregoing it appears that in *M. charantia*, 4a-phorbol-12, 13-didecanoate is hydrolyzed in situ to release the active decanoic acid probably by enzymatic process (es). Thus this plant is a store for decanoic acid. It is also reported that decanoic acid is among the three most valuable and potent antimicrobial medium chain fatty acids (MCFAs) that display antibacterial, antiviral, antifungal, anti parasitic and anti protozoal properties. The others are lauric acid and caprylic acid (Kabara, 1978). A study conducted in the Philippines showed that decanoic and lauric acids were efficient in destroying the AIDS virus (HIV) in laboratory cultures thus revealing a possible treatment for AIDS that was a lot safer and cheaper than the antiviral drugs being used at the time (Thormar, 1987). This decanoic acid component is present in ‘all important’ mothers’ milk and Coconut oil (Isaacs, 1990). Pharmaceutically, some drugs called depot injections

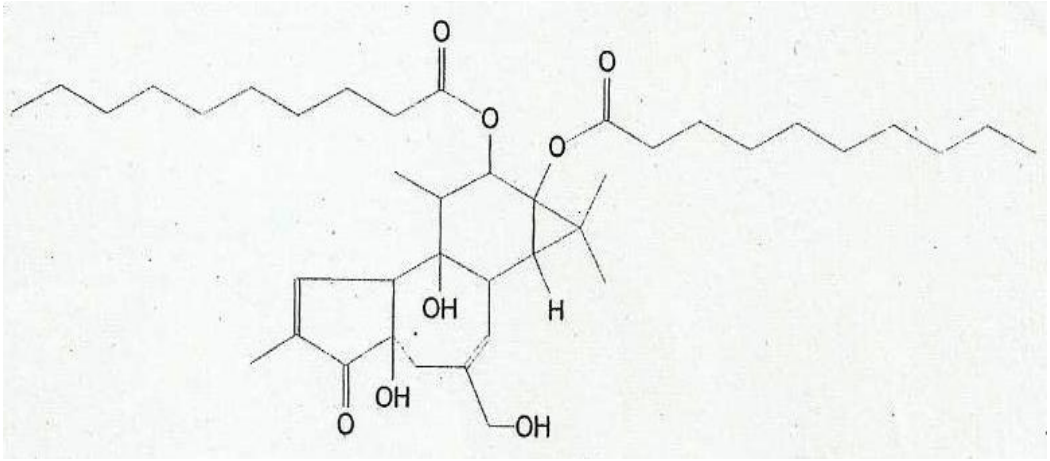


**Figure 3.** MS search result of Compound PS1- 4a-phorbol-12, 13-didecanoate.

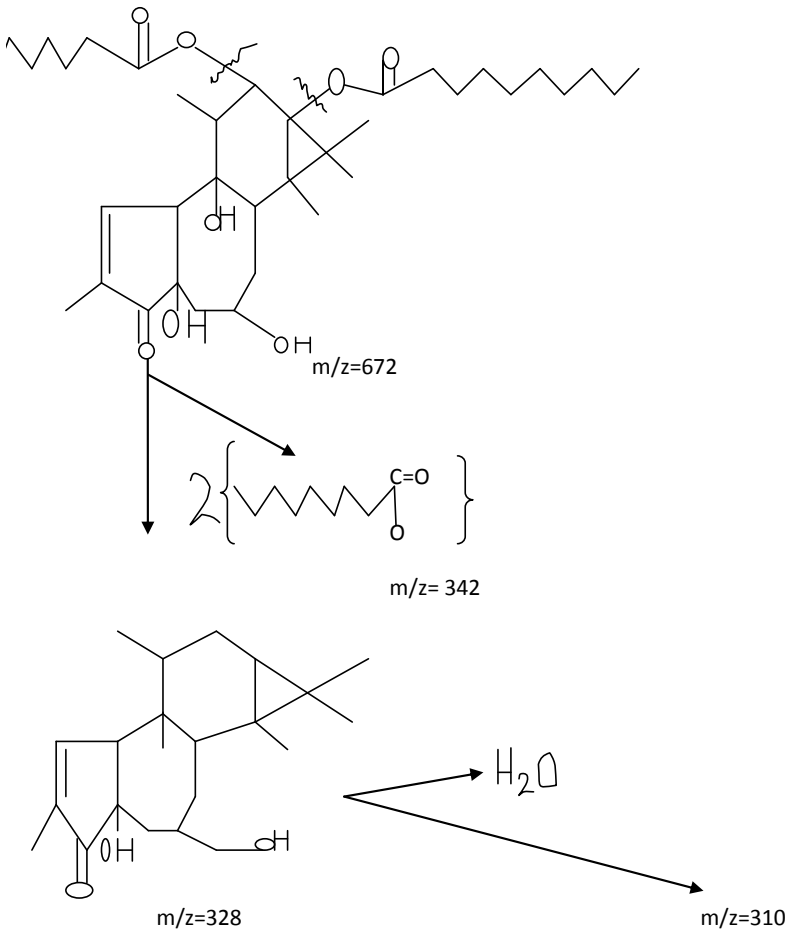


**Figure 4.** Fragmentation pattern of 4a- Phorbol-12,13-didecanoate.

contain decanoic acid in its ester form. This increases drug lipophilicity and its affinity for fatty acids because distribution of drug in fatty tissues is slow. These drugs include nandrolone used in the treatment of osteoporosis

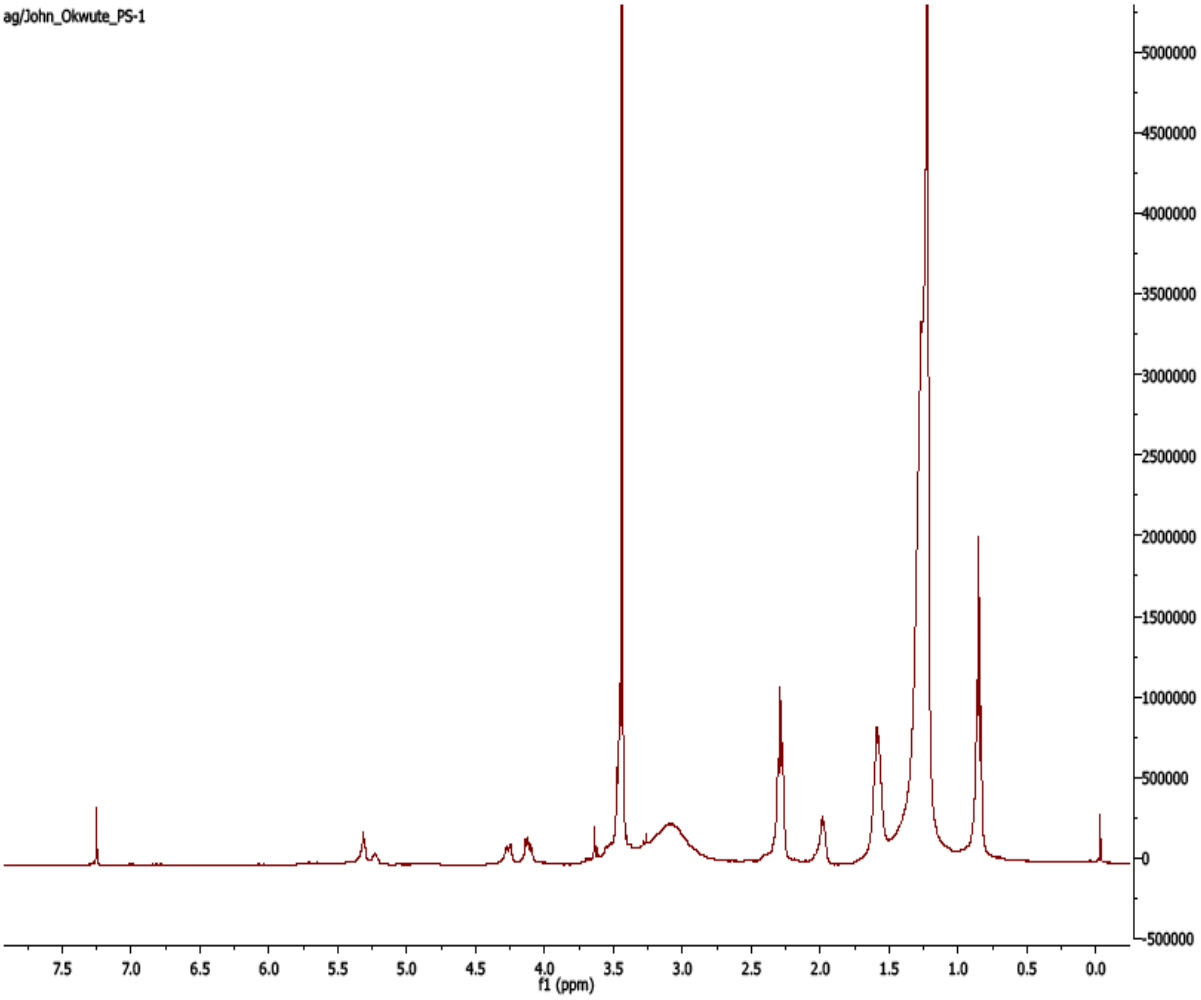


**Figure 5.** H-NMR spectrum of compound PS1.

in menopausal women and fluphenazine used for the treatment of psychoses e.g Schizophrenia. Finally, there is no past record on the isolation and characterization of 4a-Phorbol, 12, 13-didecanoate from this plant which is suggestive of this being the first. Therefore this study has contributed to the body of knowledge on phytochemicals, antimicrobials and characterization and identification of compounds in *M. charantia* seeds.

## Conclusion

We recommend that if capric or decanoic acid possesses strong antimicrobial properties by killing or inhibiting the growth of microbes such as bacteria, fungi, or viruses, the possibility of the hydrolysis of 4a-Phorbol, 12, 13-didecanoate in plant cell to release decanoic acids should be studied further for use in designing of better and safer drugs for the prevention and treatment of infections and formulation of potential disinfectants, preservatives and pharmaceuticals in food products, and in cosmetics. And also phyto-medicine should be integrated into the health system of Nigeria and developed in such a way to bring harmony between the traditional and modern system of health care with minimum threat to each other.

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