

# A 2,4,6-Trisubstituted Phenol and Trimethylsilyloxy Derivatives of Hydrocinnamic Acid and Benzene Acetic Acid from Cytotoxic Leaf Extract of *Spondias mombin* Linn (Anacardiaceae)

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## Abstract

The leaves of the Nigerian plant, *Spondias mombin*, are traditionally used in the management of inflammations and cancers. The crude methanolic extract (SM<sub>0</sub>), the hexane-soluble fraction (SM<sub>1</sub>) and the sub-fractions (SM<sub>1-1</sub>-SM<sub>1-4</sub>) of the leaves were previously investigated and reported to be cytotoxic using brine shrimps test (BST). The sub-fractions SM<sub>1-1</sub>, SM<sub>1-2</sub>, and SM<sub>1-3</sub> had LD<sub>50</sub> values at 24h as 4.63, 6.14 and 5.92 and LD<sub>90</sub> values of 41.38, 47.72 and 45.68 µg/ml, respectively. The cytotoxic fractions have now been investigated for their chemical constituents by subjecting them to chromatographic purification to give a total of 63 fractions. Ten of these fractions were found to be strongly cytotoxic and in good quantity. Further purification of fraction P29 which was partially pure using preparative TLC on silica gel gave three compounds which were characterized using GC-MS, Proton and Carbon-13 NMR spectral analyses. The compounds were identified as hydrocinnamic acid, p, alpha-bis (trimethylsilyloxy), trimethylsilyl ester, 2,6-di-tert-butyl-4-[(2-octadecyloxy carbonyl) ethyl]-phenol and benzene acetic acid, alpha-methyl-, alpha, 4-bis [(trimethylsilyl) oxy]-, methyl ester. These compounds may be responsible for the use of this plant in ethnomedicine for the management of inflammation and cancer.

**Keywords:** *Spondias mombin*; Cytotoxicity; Chemical constituents; Brine shrimps

## Introduction

Plants are used medicinally in different parts of the world as sources of many potent medicines and many of the active principles are either primary or secondary metabolites [1]. Apart from natural product-derived modern drugs, natural products are also used directly in the pharmaceutical industries in Europe and North America. In traditional medicine programmes they are being incorporated into the primary healthcare system of some countries including Mexico, the Republic of China,

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Nigeria and other developing countries [2]. It is well known that cancer is second to cardiovascular disease as a natural cause of death and most of the synthetic chemical agents currently being used in cancer therapy are toxic and cause damage to normal cells [3]. Many plants have therefore been investigated in order to identify new effective and safe antioxidant and anticancer compounds, as well as study their mode of action of cancer cell inhibition [3].

In our continued search for bioactive natural products, including potential anticancer agents, we have recently screened and reported the cytotoxicity of a number of Nigerian plants traditionally used to manage inflammations and tumors [4-6]. In this study the cytotoxic hexane-soluble fraction of the crude extract of the leaves of *Spondias mombin* [4] was subjected to chromatographic purification to identify some of its active chemical components. We now wish to report the cytotoxicity, isolation and characterization of some rather unusual chemical constituents from the leaves of *Spondias mombin*.

## Materials and Methods

### Materials

The leaves of *Spondias mombin* were collected from Odo-Owa, Oke Ero Local Government of Kwara State. The plant, *Spondias mombin*, was identified by Mr. B. E. Omomoh, a taxonomist at the Department of Botany, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria with voucher specimen number IFE17284. The leaves were air-dried at room temperature in the Chemistry laboratory of Federal University of Technology Minna, Niger State and milled into powder using a blender.

All solvents and chemicals used in this work were of standard grade and were appropriately purified before use. Preparative TLC plates were prepared using 30 g silica gel impregnated with floreicin and 60 ml of distilled water for five 20 × 20 cm plates. The plates were allowed to dry at room temperature and then activated at 110 °C before use. They were viewed using UV lamp model UVGL-15 at 254 nm and 365 nm. GC-MS analysis was performed using Agilent Technologies 6890N Network GC system coupled with Mass selective Detector 5975 apparatus. The carrier gas was helium and the column temperature was 280 °C and the flow rate was 1 µL/min. The MS source was at 240 °C and the plunger speed was fast. Proton and 13C nmr spectra were obtained on Bruker Avance III HD 400 MHz NMR with 5 mm BBO probe. A 20 mg sample was dissolved in 500 µL DMSO-d<sub>6</sub> solvent. The <sup>1</sup>H NMR spectra were acquired at 400 MHz and the spectra were calibrated at 2.5 ppm for the DMSO peak while the <sup>13</sup>C NMR spectra were acquired at 100 MHz and calibrated at 39.51 ppm for the DMSO solvent peak.

### Methods

**Extraction of plant material and fractionation of crude extract:** The powdered leaf of *Spondias mombin* was cold extracted with methanol (1L × 6 days) and the extract was concentrated *invacuo* to dryness. The crude extract, SM<sub>0</sub>, was dissolved in 100 ml of distilled water and partitioned with n-hexane to give the corresponding fraction SM<sub>1</sub>. The hexane fraction was subjected to VLC to give the sub-fractions SM<sub>1-1</sub> to SM<sub>1-4</sub>. The crude extract, hexane fraction and the sub-fractions were screened for cytotoxicity using BST bioassay as previously reported [4].

**Isolation and characterization of compounds:** The sub-fractions, SM<sub>1-1</sub>, SM<sub>1-2</sub>, and SM<sub>1-3</sub> arising from VLC using hexane and hexane/chloroform as solvents were further purified by column chromatography on silica gel 60G using hexane, hexane-chloroform, chloroform/methanol and methanol as eluents to give fractions, CC1-CC7. The column fractions CC1, CC3 and

CC5 were further subjected to column chromatography, followed by repeated preparative thin-layer chromatography to give 63 sub-fractions, P1-P63. Those in reasonable quantity were screened for Brine Shrimps cytotoxicity. Fraction P29 was partially pure and was further purified to give three isolates, P29A, P29B and P29H which were subjected to GC-MS,  $^1\text{H}$  and  $^{13}\text{C}$  NMR analyses.

## Results and Discussion

The crude methanolic extract of the leaves of *Spondias mombin*, the hexane fraction and the hexane sub-fractions were shown to be cytotoxic in BST bioassay as previously reported [4]. The hexane sub-fractions  $\text{SM}_{1-1}$ ,  $\text{SM}_{1-2}$ , and  $\text{SM}_{1-3}$  were promising in terms of cytotoxicity and were further subjected to column chromatography and BST bioassay. The results are shown in TABLES 1 and 2. The column fractions CC1, CC3 and CC5 were further subjected to column chromatographic purification on silica gel to give 63 sub-fractions of which the ten most significant in terms of quantity and toxicity are shown in TABLE 3. The sub-fractions were generally impure but P29 was in good quantity and was partially pure. It was then purified by repeated preparative TLC to give the isolates, P29A, P29B and P29H which were then subjected to GCMS,  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral analyses. The GC of P29A (FIG. 1)

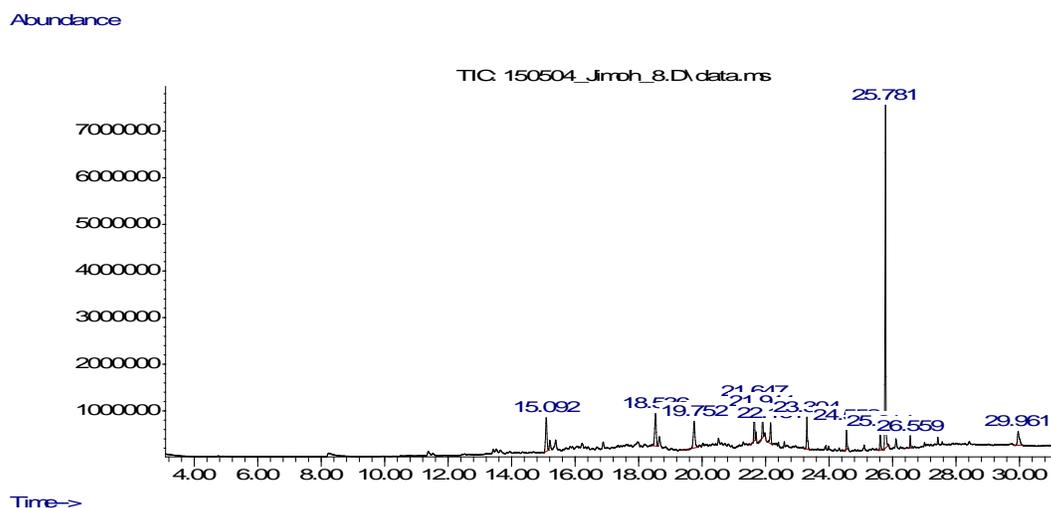


FIG. 1. Gas chromatogram of compound P29A.

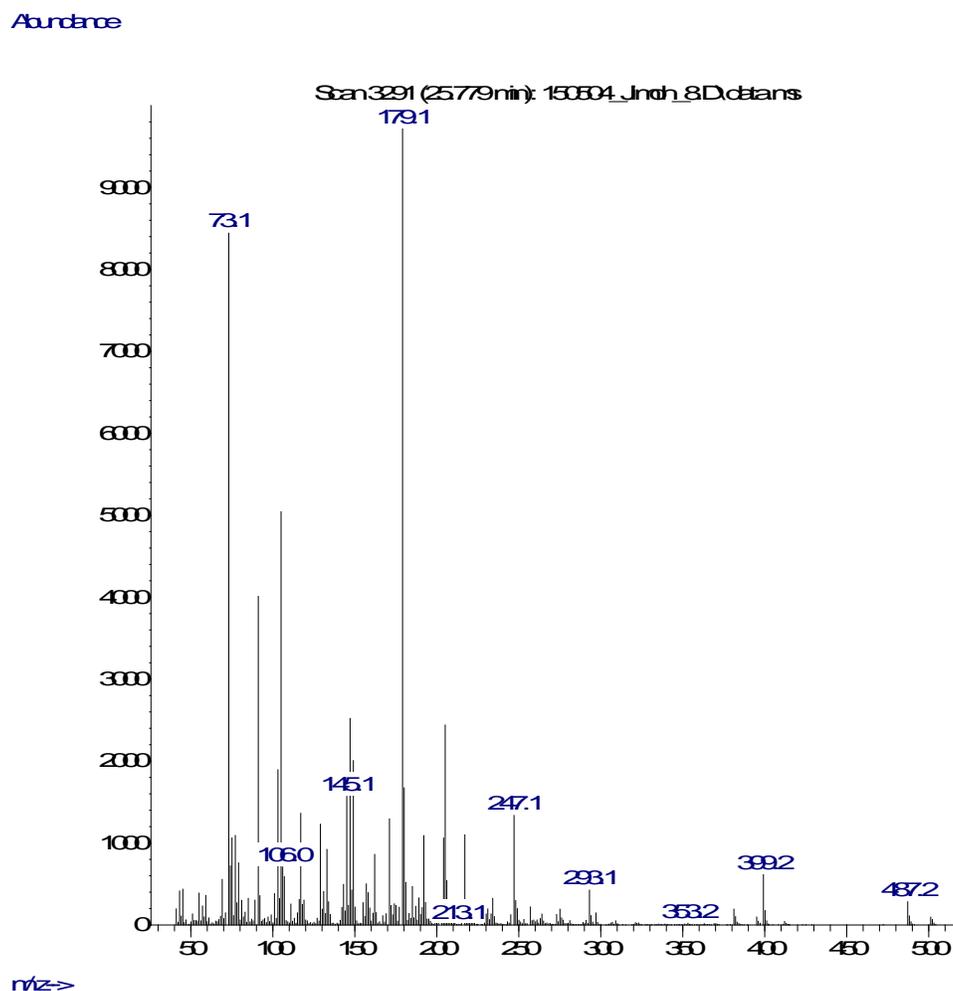


FIG. 2. Mass spectrum of compound P29A.

TABLE 1. Results of cytotoxicity screening of crude methanolic extract and the VLC sub-fractions of the hexane fraction against *Artemiasalina*.

Samples	LD50 $\mu\text{g/ml}$	LD90 $\mu\text{g/ml}$
SM <sub>0</sub>	29.17 $\pm$ 3.18	115.12 $\pm$ 7.83
SM <sub>1</sub>	3.00 $\pm$ 0.066	13.39 $\pm$ 1.89
SM <sub>1-1</sub>	4.63 $\pm$ 0.76	41.38 $\pm$ 3.71
SM <sub>1-2</sub>	6.14 $\pm$ 0.45	47.07 $\pm$ 1.31
SM <sub>1-3</sub>	5.92 $\pm$ 0.29	45.68 $\pm$ 2.15
SM <sub>1-4</sub>	1.24 $\times$ 10 <sup>4</sup> $\pm$ 00	1,12 $\times$ 10 <sup>5</sup> $\pm$ 1.12 $\times$ 10 <sup>5</sup>

Showed a major peak at retention time (RT) 25.787 minutes. The MS (FIG. 2) gave a base peak at  $m/z$  179.1 and a molecular ion peak at 399.2 ( $M^+H$ ). The proton nmr spectrum revealed the presence of aromatic proton signals at 7.2. An oxymethine proton signal was observed at  $\delta$  5.7-5.8 as a quartet due to coupling with the methylene group at C-2. This was associated with a doublet signal at 4.48-4.90 for the methylene group at C-3. No proton signal for a phenolic hydroxy group was recorded at about 5.2, showing that there was no free phenolic OH function in the molecule. The signals at 0.81-0.87, 2.18-2.34 and 1.99 -2.04 support the presence of a trimethylsilyloxy ester and two trimethylsilyloxy groups in the molecule.

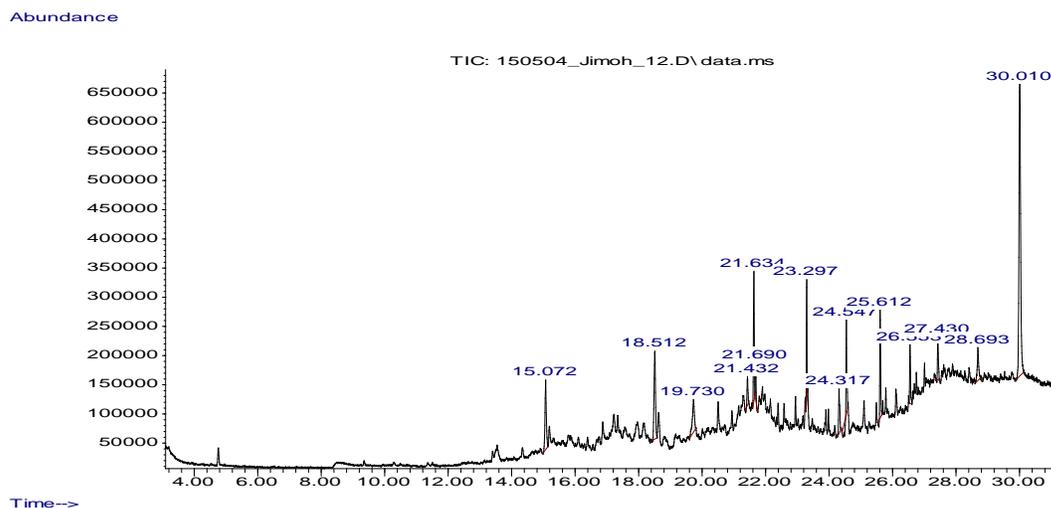


FIG. 3. Gas chromatogram of compound P29B.

TABLE 2. Results of toxicity screening of column chromatographic fractions of SM<sub>1-1</sub>.

Column fractions	LD50 $\mu\text{g/ml}$	LD90 $\mu\text{g/ml}$	Wt gm
CC1	3.72 $\pm$ 1.01b	1001 $\pm$ 240, 35a	1.1845
CC2a	9.72 $\pm$ 6.22ab	1.09 $\times$ 106 $\pm$ 91.06b	0.1652
CC2b	12.96 $\pm$ 4.85ab	587.61 $\pm$ 53.24ab	0.3389
CC3	4.26 $\pm$ 0.33b	33.32 $\pm$ 0.78b	0.3143
CC4	3.20 $\pm$ 0.24b	24.55 $\pm$ 1.97b	0.14
CC5	16.11 $\pm$ 4.65a	53.99 $\pm$ 7.02b	0.5968
CC6	5.31 $\pm$ 0.29ab	38.54 $\pm$ 0.39b	0.257
CC7	9.58 $\pm$ 4.51ab	40.81 $\pm$ 7.81b	0.5523

TABLE 3. Ten most cytotoxic chromatographic fractions.

Code of isolate	LD50 $\mu\text{g/ml}$	LD90 $\mu\text{g/ml}$	Wt (mg)
P23.	$1.38 \pm 0.27\text{cd}$	$6.64 \pm 0.90\text{b}$	26.2
P24	$1.77 \pm 0.12\text{cd}$	$8.05 \pm 0.31\text{b}$	49.2
P29	$1.57 \pm 0.44\text{cd}$	$7.42 \pm 1.51\text{b}$	174.5
P38	$0.85 \pm 0.08\text{d}$	$5.10 \pm 0.32\text{b}$	77
P43	$2.13 \pm 0.36\text{bc}$	$35.61 \pm 10.75\text{b}$	20
P47	$3.37 \pm 1.02\text{bc}$	$25.99 \pm 2.55\text{b}$	50
P48	$3.33 \pm 0.62\text{bc}$	$28.29 \pm 4.43\text{b}$	62.1
P49	$1.83 \pm 0.30\text{cd}$	$13.59 \pm 4.37\text{b}$	57.6
P58	$7.14 \pm 1.92\text{a}$	$535.11 \pm 102.99\text{a}$	19
P59	$4.15 \pm 0.21\text{b}$	$15.61 \pm 0.46\text{b}$	59.9

The  $^{13}\text{C}$  NMR signal at 173 is indicative of a cinnamic acid ester carbonyl carbon while the peaks at 139.3 and 114.0 account for 6 aromatic carbons in good agreement with NMR spectral data for hydroxycinnamic acids such as coumaric and chlorogenic acids [7-9] but there is no evidence of the presence of ethylenic carbons. This suggests that P29A may be a hydrocinnamic acid derivative. The other groups of carbon signals at 30.0-31.9, 29.6-29.7 and 28.9-29.5 may be due to the trimethylsilyloxy carbons. Thus, although the  $^{13}\text{C}$  NMR spectrum showed 12 carbons the molecular mass of 399.2 suggests that there are about four sets of equivalent carbons, the trimethylsilyloxy and the aromatic ring carbons. The proton and carbon NMR spectral data are presented in TABLE 4. Compound P29A is probably hydrocinnamic acid, p, alpha-bis (trimethylsilyloxy), trimethylsilyl ester (FIG. 4 and 5), based on the above spectral characteristics and standard MS library spectral data.

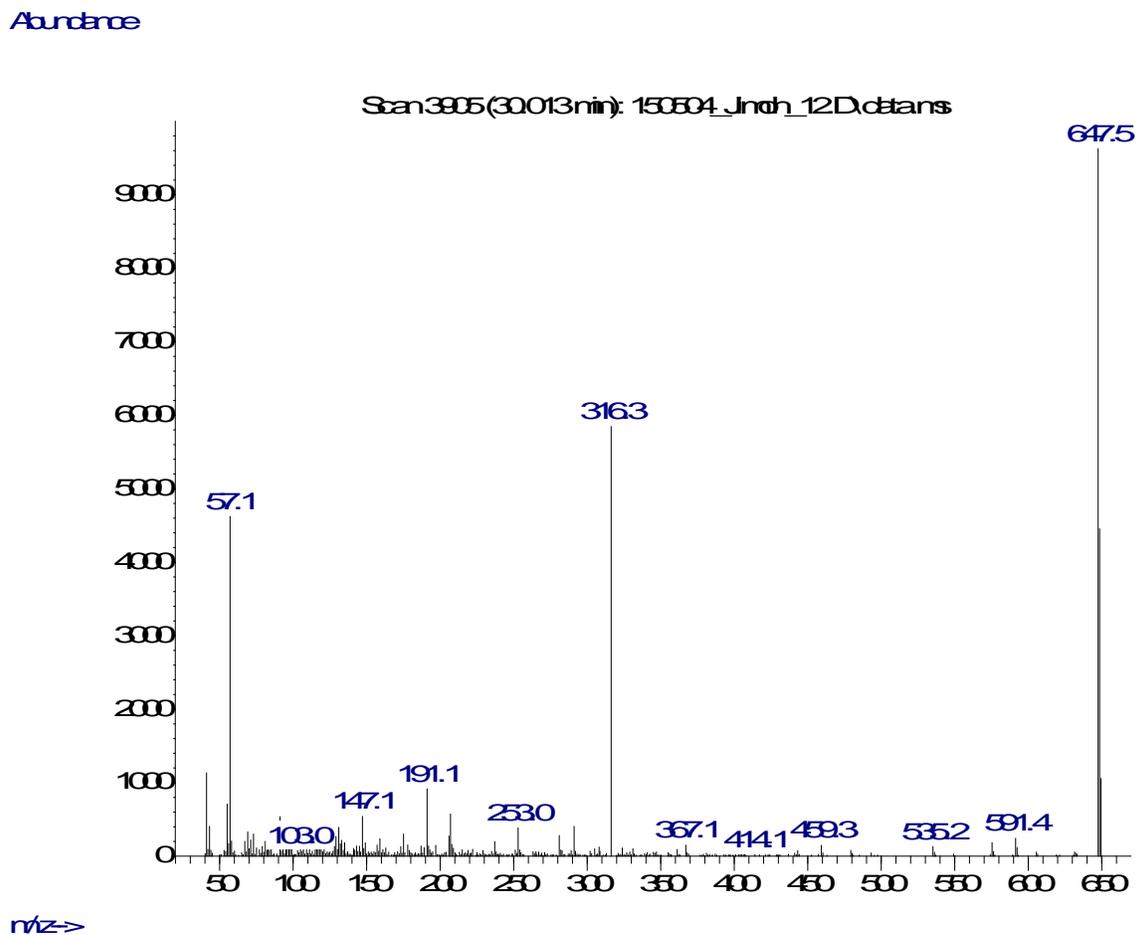


FIG. 4. Mass spectrum of compound P29B

The GC of P29B (FIG. 3) showed a major peak with retention time of 30.010 minutes. The MS of P29B (FIG. 4) showed a fragment ion peak at  $m/z$  57.1 which corresponds to elimination of propenal  $[\text{CH}(\text{O})-\text{CH}=\text{CH}_2]^+$  group [10]. In addition, the MS showed a base peak at  $m/z$  316 due to  $[\text{CH}_3(\text{CH}_2)_{16}\text{CH}(\text{CH}_3)-\text{O}-\text{CO}-\text{CH}-\text{CH}_2]^+$  fragment ion and a molecular ion peak at  $m/z$  530. The  $^{13}\text{C}$  nmr spectrum showed an ester carbonyl peak at 173 but did not give strong signals for aromatic carbons. However, the  $^1\text{H}$  nmr spectrum revealed the presence of aromatic proton signals at  $\delta$ 7.2. The presence of a proton signal at  $\delta$ 5.27 suggested the presence of a phenolic function. The complex resonance peak at  $\delta$ 4.89 -4.99(q) is probably due to the methine proton while the triplet at  $\delta$ 0.83-0.85 is due to the terminal methyl group of a long aliphatic chain. There are also other methyl proton signals at  $\delta$ 1.99-2.07 and methylene signals between  $\delta$ 1.23 and  $\delta$ 1.55 [11]. The  $^{13}\text{C}$  nmr spectrum showed prominent aliphatic chain carbon peaks at 14.12, 22.7, 27.7 - 29.37 and 31.9 ppm. These spectral data (TABLE 5) and the standard MS library data support the fact that P29B is probably 2, 6-di-tert-butyl-4-[(2-octadecyloxycarbonyl) ethyl]-phenol (FIG. 6).

TABLE 4.  $^1\text{H}$  nmr and  $^{13}\text{C}$  nmr spectral data of compound P29A.

Carbon	$^1\text{H}$ (ppm)	$^{13}\text{C}$ (ppm)	Assignment
1	-	173	>C=O
2	5.7-5.8	37.4	>CH-O-
3	4.9-4.48	37.1	-CH <sub>2</sub> -
4	-	114	Aromatic
5	7.2	114	Aromatic
6	7.2	114	Aromatic
7	7.2	139	Aromatic
8	7.2	114	Aromatic
9	7.2	114	Aromatic
10.a/b/c	0.81-0.87	30.0-31.9	-O-Si [CH <sub>3</sub> ] <sub>3</sub> (methyl)
11.a'/b'/c'	1.99-2.04	28.9-29.5	O=C-O-Si [CH <sub>3</sub> ] <sub>3</sub> (methyl)
12.a''/b''/c''	2.20-2.30	29.6-29.7	Ar-O-Si[CH <sub>3</sub> ] <sub>3</sub> (methyl)

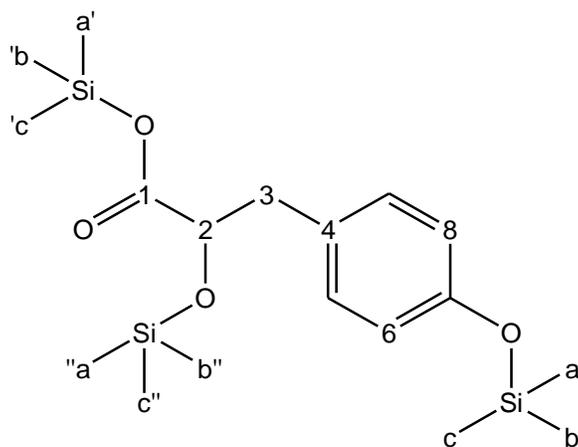
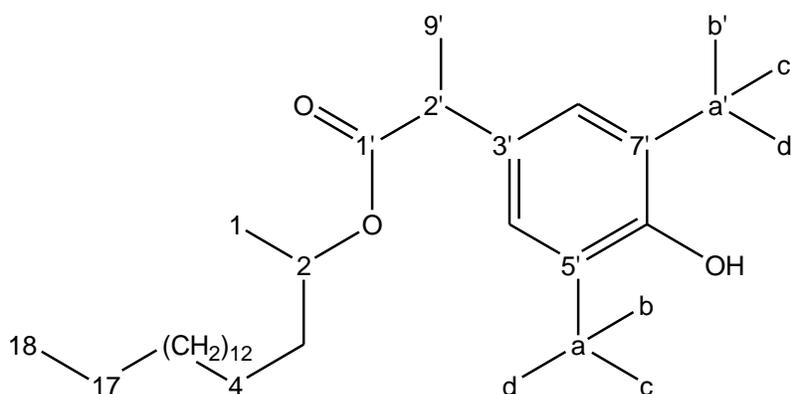
FIG. 5. (a, b, c=CH<sub>3</sub>).

TABLE 5. The  $^1\text{H}$  and  $^{13}\text{C}$  nmr spectral data of Compound P29B.

Carbon	$^1\text{H}$ (ppm)	$^{13}\text{C}$	Assignment
1	1.26	22.7	-CH <sub>3</sub> group
2	5.7-5.8	76.0	-oxymethine
3	4.3	31.6	-CH <sub>2</sub> -
4	2.9	22.7	-C H <sub>2</sub> -
5-16	1.5-2.5	29.3-31.9	-CH <sub>2</sub> -
17	2.6	22.7	-CH <sub>2</sub> -
18	0.85	14.2	0
1'	-	173.0	carbonyl carbon
2'	4.3	43.0	methine
3'	-	149.0	Aromatic
4''	7.2	124.9	Aromatic
5'	-	134.1	Aromatic
6'	5.0(OH)	149.0	Phenolic
7'	-	134.1	Aromatic
8'	-	124.9	Aromatic
9'	1.9	22.7	0
a/a'	-	32.0	Ar-butyl carbon
b/b'	1.5	31.93	Ar-C(CH <sub>3</sub> ) <sub>3</sub> methyl
c/c'	1.5	31.93	“
d/d'	1.5	31.93	“

FIG. 6. (a=C), (9, 18, b, c, d=CH<sub>3</sub>)

The GC of P29H (FIG. 7 and 8) showed a major peak with a retention time of 25.776 minutes. The MS of this component gave a molecular ion peak at  $m/z$  399 representing the protonated molecule. The other significant fragment ions included  $m/z$  73.1 for  $[\text{Si}(\text{CH}_3)_3]^+$  and  $m/z$  117 for  $[\text{CO}-\text{O}-\text{Si}(\text{CH}_3)_3]^+$ . The cleavage of two trimethyl silyl groups and the cleavage of the carbon-carbon bond alpha to the ester carbonyl group gave  $[\text{O}=\text{Ar}=\text{CH}-\text{CO}-\text{CH}_2]^+$  corresponding to the peak at  $m/z$  145.1. The peak at  $m/z$  179.1 was the base peak due to  $[\text{Si}(\text{CH}_3)_3-\text{O}-\text{Ar}-(\text{CH}_2)]^+$  fragment ion. The  $^1\text{H}$  nmr spectrum of P29H revealed the presence of four aromatic protons signal at  $\delta$ 6.97, 7.099, 7.11 and 7.24. The chemical shift at  $\delta$ 4.89-4.99 was due to a methoxyl group while those at  $\delta$ 2.26-2.38 and  $\delta$ 1.99-2.04 are due to trimethylsilyl protons (18H). The  $^{13}\text{C}$  nmr revealed

aromatic carbons signals at 139 and 114 while the aliphatic carbons are at 37.8, 31.9 and 29 ppm. The above spectral characteristics and the library MS data were suggestive that compound P29H has the structure (FIG. 9). The nmr spectral data are shown in TABLE 6.

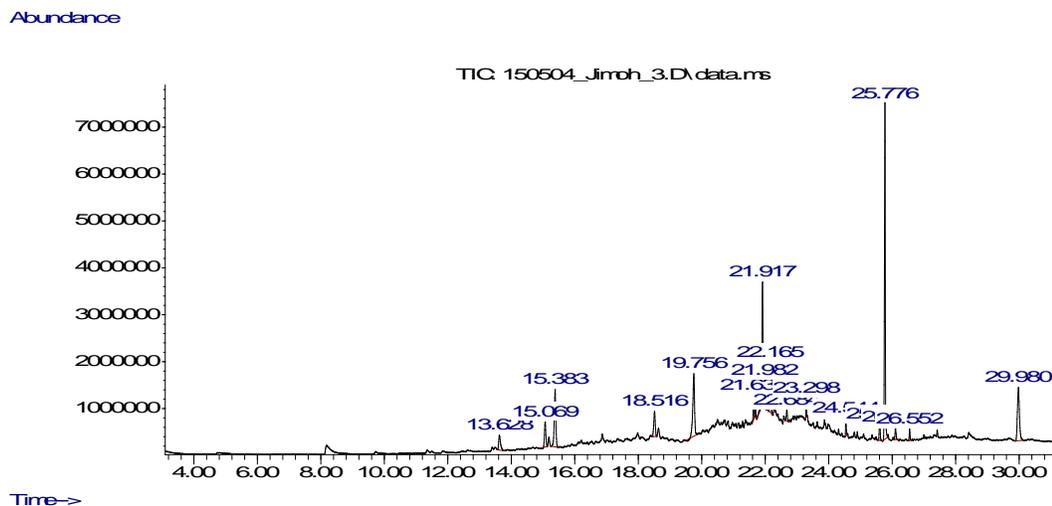


FIG. 7. Gas chromatogram of compound P29H.

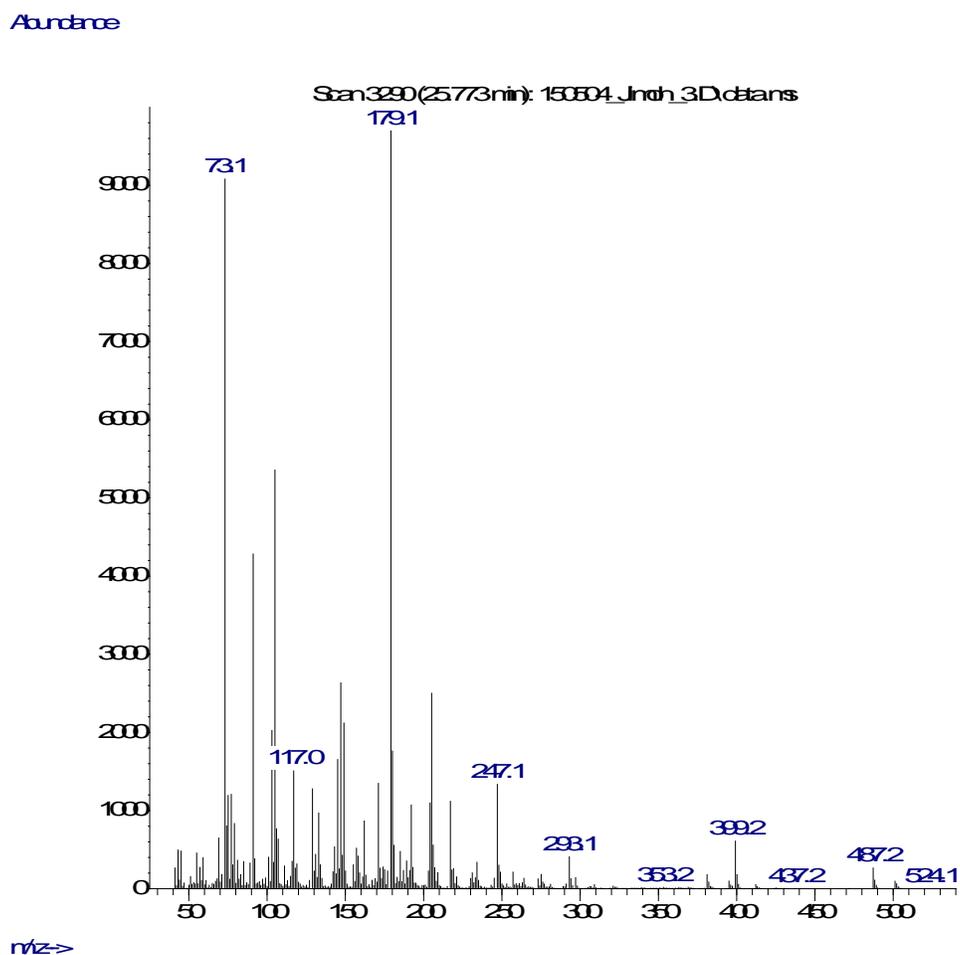


FIG. 8. Mass spectrum of compound P29H

TABLE 6.  $^1\text{H}$  and  $^{13}\text{C}$  nmr spectral data of compound P29H

1	Nil	172.0	Carbonyl
2	-	61.78	Quarternary
3	-	"	Aromatic
4	7.23(dd)	"	Aromatic
5	7.11(dd)	"	Aromatic
6	-	"	Aromatic
7	7.11	61.78	Aromatic
8	7.23	61.78	Aromatic
9	4.89-4.99	41.0	Methyl
10	5.70-5.80	59.76	Methoxy
11a/b/c	2.26-2.37	14.0, 22.0, 26.0	Trimethylsiloxy
12a'/b'/c'	1.99-2.00	29.0, 31.9, 37.8	Trimethylsiloxy

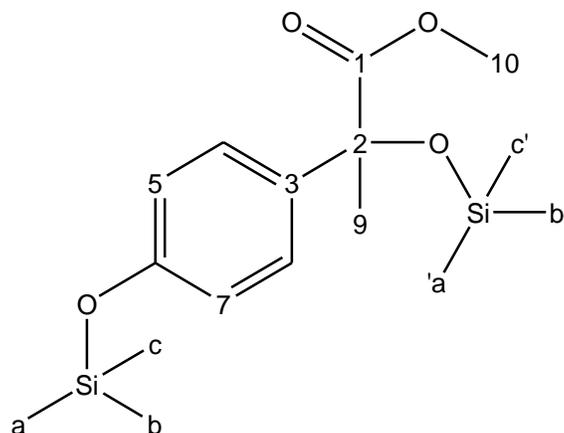


FIG. 9. Methyl Ester.

Thus, the chemical investigation of the cytotoxic hexane-soluble fraction of the leaves extract of *Spondias mombin* has led to the isolation and characterization of three compounds which are new to the genus *Spondias* and the family, Anacardiaceae. Cinnamic acid and its phenolic analogues are natural compounds which have long been associated with anticancer activity and were first put into clinical use in 1905 [7]. They are known to be of interest due to their antioxidant, anti-inflammatory and anticarcinogenic activities [8,9]. Cinnamic acid derivatives are relatively common in plants and their biogenetic origin established [7], but their trimethylsilyloxy derivatives are very rare in nature. However, the trimethylsilyl derivatives of natural non-volatile hydroxycinnamic acids have been prepared and used to analyse them using GC-MS [12]. Also, two related natural organic silicon compounds, 1,1,1,5,7,7,7, heptamethyl 3,3 (trimethylsilyloxy) tetrasiloxane and 1,1,1,3,5,7,7,7, octamethyl-3,3-bis (trimethylsilyloxy) tetrasiloxane have also been reported as oxidative degradation products of sporopollenin extracted from the pollens and spores of *Magnolia grandiflora* (Magnoliaceae) and *Hibiscus syriacus* (Malvaceae) using GC-MS to analyse the hexane extract [13]. Previously benzene acetic acid, 4-bis [(trimethylsilyl)oxy] - trimethylsilyl ester, which is closely related to benzene acetic acid, alpha, -methyl-, alpha, 4-bis [(trimethylsilyl) oxy]-, methyl ester (FIG. 9) had been identified from *Actepila excelsa* as an antioxidant and cancer preventive agent [14].

## Conclusion

This work has succeeded in isolating and characterising three trimethylsilyloxy derivatives of some natural phenolic compounds new to the family, Anacardiaceae and the genus, *Spondias*. These phytochemicals may be responsible for the toxicity observed in this work and for the ethno medicinal application of the plant in the management of inflammations and cancers.

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## REFERENCES

1. Kubmarawa D, Ajoku GA, Enwerem NM, et al. Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. African Journal of Biotechnology. 2007; 6: 1690-96.

2. Satyajit D, Sarker ZL, Alexander IG. Methods in biotechnology: Natural product isolation, second edition. 2006; 4-6.
3. Hussein AS, Hassan MF, Madkour LA, et al. Antioxidant and cytotoxic activities of *Gmelina arborea* ROXB leaves. British Journal of Pharmaceutical Research. 2014; 4: 125-44.
4. Ibikunle GF, Okwute SK, Ogbadoyi EO. Cytotoxic agents from Nigerian plants: A case study of *Spondias mombin* Linn (Anacardiaceae) leaves. FUW Trends in Science and Technology Journal. 2017; 2: 510-13.
5. Okwute SK, Ochi IO. Phytochemical analysis and cytotoxic activity of the root extract of *Commiphora Africana* (Caesalpiniaceae). Journal of Pharmacognosy and Phytochemistry. 2017; 6: 451-54.
6. Okwute SK, Okoh-Esene RU, Okogun JI. Phytochemical, antimicrobial and anti-epilepsy characteristics of the root of *Hippocratea welwitschii* (Celastraceae). International Journal of Pharmacy and Pharmaceutical Research. 2017.
7. De P, Baltas M, Bedos-Belval F. Cinnamic acid derivatives as anticancer agents-A review Current Medicinal Chemistry. 2011; 18: 1672-1703.
8. Strack D, Leicht P, Bokem M, et al. Hydroxycinnamic acid esters of isocitric acid: Accumulation and enzymatic synthesis in *Amaranthus cruentus*. Phytochemistry. 1987; 26: 2919-22.
9. Schroter D. Natural diversity of hydroxycinnamic acid derivatives, flavonoid glycosides, carotenoids and chlorophylls in leaves of six different Amaranth species. Food Chemistry.
10. Allen F, Greiner R. Comparative fragmentation modeling of ES1-MS/MS spectra for putative metabolite identification. Metabolomics. 2015; 11: 98-110.
11. Silverstein RM, Bassler GC, Morrill TC. Spectrometric identification of organic compounds. 1991.
12. Castele KV, Pooter HD, Sumere CFV. Gas chromatographic separation and analysis of trimethylsilyl derivatives of some naturally occurring non-volatile phenolic compounds and related substances. Journal of Chromatography. 1976; 121: 49-63.
13. Masaya K, Masamichi T. Gas chromatography-Mass Spectrometric analysis of oxidative degradation Products of Sporopollenin in *Magnolia grandiflora* (Magnoliaceae) and *Hibiscus syriacus* (Malvaceae). Journal of Plant Research. 1996; 109: 297-299.
14. Ramesh P, Akalanka D. Bioactive constituents of *Actephila excels* (dalz). Traditional medicinal plants. International Journal of Chemical and Pharmaceutical Sciences. 2014; 5: 11-16.