

**Isolated Compounds from the Roots of *Flabellaria paniculata* Cav. (Malpighiaceae) and their Effects on MCF-7 Breast Cancer Cells**Oluwatosin O. Johnson<sup>1\*</sup>, Sarita G. Bhat<sup>2</sup>, Gloria A. Ayoola<sup>1</sup>, Harikrishnan Madayath<sup>2</sup>, Saipriya P. Puthusseri<sup>2</sup>, Herbert Coker<sup>1</sup><sup>1</sup>Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Lagos, CMUL Campus, Lagos 100254, Nigeria<sup>2</sup>Department of Biotechnology, Cochin University of Science and Technology, Kochi, South Kalamassery, Kerala, 680022, India

## ARTICLE INFO

## ABSTRACT

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*Flabellaria paniculata* Cav. (Malpighiaceae) has been used for various medicinal purposes in West Africa. In southwest Nigeria, the roots are being used by traditional and Ayurveda practitioners for the treatment of breast cancer. This study was carried out on the investigation of *Flabellaria paniculata* roots, by establishing the inhibitory concentration of the crude extract, fractions and identifying possible compounds that may be responsible for the treatment or prevention of breast cancer in Nigeria. Crude methanol extract was obtained by percolation method. The compounds were isolated and purified by analytical thin layer chromatography (TLC) and column chromatography (CC). Isolated compounds were characterized using Nuclear Magnetic Resonance (NMR), High Resolution Mass Spectrometry (HRMS) and Fourier Transform Infrared (FTIR). *In-vitro* activity was done by MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide) assay using MCF-7 breast cancer cells line. Two known phytosterols were isolated from *F. paniculata* roots; Campesterol glucoside (1) and Sitosterol (2). Campesterol glucoside with ( $IC_{50}$  1.18±0.18 mg/mL) showed better activity than Sitosterol (1.79±0.14 mg/mL). The activities were less when compared to a standard anticancer drug, Paclitaxel ( $IC_{50}$  0.07±0.03 mg/mL). The two compounds were more active than a well-known flavonoid, Quercetin with ( $IC_{50}$  2.05±0.16). The hexane fraction (0.97±0.05 mg/mL) was the most active among the fractions (ethylacetate 2.97±0.19, butanol 6.22±0.17 and water 4.10±0.13 mg/mL) tested. The isolated compounds may reveal the reason for the traditional use of this medicinal plant in breast cancer treatment in Southwest Nigeria and West Africa. Therefore, Campesterol glucoside may be exploited further for its potency clinically.

**Keywords:** Breast cancer, Campesterol glucoside, *Flabellaria paniculata*, Sitosterol, Spectroscopy.

**Introduction**

*Flabellaria paniculata* Cav. belongs to the family Malpighiaceae. The medicinal plant is native to tropical Africa. It is a climbing shrub with 3-15 meters in height.<sup>1</sup> The leaves and roots of *F. paniculata* are used as a traditional herb in West Africa, most especially in Nigeria, Ghana, and Ivory Coast, for treating diarrhea, dysentery, sores and wounds, snake immunization, and breast cancer.<sup>2-5</sup> The previous study revealed the chemical constituent and anti-inflammatory properties of the essential oil from the leaves and the roots of *F. paniculata* which was dominated by the presence of sesquiterpenoids (69.9%) and diterpenes (84.1%) respectively.<sup>6</sup> Recently, the following compounds were reported to be isolated from the leaves of *F. paniculata*; steroids (sitosterol- $\beta$ -d-glucoside and sitosterol), triterpenoids (friedelinol and friedelin), and a flavonoid glycoside (kaempferol-3-O- $\alpha$ -l-rhamnopyranosyl-(1→6)- $\beta$ -d-glucopyranoside).<sup>7</sup>

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Female breast cancer is listed as the fifth leading cause of death (627, 000 deaths), because the prognosis is relatively favorable, at least in more developed countries. In Nigeria, the number of new cases in 2018, both sexes, all ages was 26,310 (22.7%) out of 115,912 total new cancer cases, this resulted in 11,564 deaths (16%). Therefore, breast cancer was ranked number one in the cause of death from cancer. While in India, the number of new cases in 2018, both sexes, all ages was 162,468 (14%) from 1,157,294 total new cancer cases, this resulted in 87,090 deaths (11.1%).<sup>8</sup>

Although the roots and leaves of *F. paniculata* are used for treating breast cancer by the traditional medical practitioners in southwest Nigeria, no scientific documentation of this claim has been reported. Hence, this study was to investigate *F. paniculata* roots and established its inhibitory concentration of the extracts, fractions and identifying possible compounds responsible for its breast cancer treatment.

**Materials and Methods***Instrumentation*

Infrared spectra were measured on a Nicolet™ iS50 FTIR Spectrometer (Thermo scientific), featuring purpose-built accessories and integrated software. Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker Advance III HD 400 MHz one bay FT-NMR spectrometer. All chemical shifts were quoted on the chemical ( $\delta$ ) scale in ppm using residual solvent as the internal standard, Dimethyl sulfoxide (DMSO-*d*<sub>6</sub>: 2.5 ppm for <sup>1</sup>H-NMR, 39.5 ppm for <sup>13</sup>C-NMR; CDCl<sub>3</sub>: 7.24 ppm for <sup>1</sup>H-NMR, 77.0 ppm for <sup>13</sup>C-NMR). Coupling constants (*J*) are reported in Hertz (Hz). High Resolution Electrospray Ionization Mass Spectrum was measured on Thermo

Fisher Scientific (Exact model) using positive electrospray ionization. Absorbance reading for MTT (dye compound, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was determined using Life science microplate reader (TECAN®).

#### Collection of plant materials

Fresh roots of *F. paniculata* were collected from Agbara area of Ado-Odo, Ota Local Government, Ogun State, Southwest Nigeria in April 2019. Roots material was authenticated by Mr. G. I. Nodza of the Department of Botany, Faculty of Science, University of Lagos, Nigeria and deposited in the herbarium of the same department with a voucher specimen number LUH 8368. The fresh roots (4.65 kg) were washed under running tap water to remove sand and debris, was air-dried under shade for 14 days. Final weight after drying was 2.4 kg, and powdered using a mechanical grinder.

#### Extraction and isolation of components

The powdered air-dried roots of *F. paniculata* (2.25 kg) were extracted with methanol (5 L) at room temperature using percolation method. The continuous extraction was done for 7 days and it yielded 127.16 g of dried crude extract. The crude extract (123.16 g) was partitioned with the aid of a separating funnel into hexane-soluble (17.71 g), ethyl acetate soluble (15.46 g), *n*-butanol-soluble (25.68 g) and water fractions (61.31 g). The hexane-soluble fraction (15.61 g) was subjected to column chromatography on silica gel (60 -120 mesh, 150 g, column size 60 × 3 cm) and eluted with mixtures of hexane - ethylacetate (100:0 to 50:50 v/v, gradient elution). This afforded ten fractions and by TLC monitoring using hexane/ethylacetate as solvent system, fractions were combined into five fractions (Fraction A–E). Fraction E (4.92 g) which showed a distinct separation on the analytical TLC plate was further separated on a silica gel column (60 – 120 mesh, Column size 45 × 3 cm), using hexane – acetone (100:0 to 40:60 v/v) as mobile phase, compound **1** precipitated out at fraction 80:20 and compound **2** precipitated out at 40:60. Compound **1** was further purified on a silica gel column (60 – 120 mesh, 20 g, column size 30 × 2 cm), using dichloromethane (DCM) – methanol (98:2, isocratic elution) as mobile phase. This finally gave 51 mg. Compound **2** was further purified over silica gel column (60 – 120 mesh, 17g, 30 × 2 cm), using chloroform-methanol (95:5, isocratic elution) as mobile phase. This finally gave 52 mg. All compounds isolated were kept in a vacuum desiccator containing a dehydrating agent; concentrated sulphuric acid and silica gel to remove any solvent prior to spectroscopic data collection.

#### Biological assay

##### Cell line culture

Human breast cancer cells (MCF-7) were obtained from Amala cancer research centre, Amala, Nigar, Thrissur, 680555, Kerala, India. The MCF-7 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), (100U) 20µg/mL penicillin, and 100 µg/mL streptomycin. Incubation was carried out at 37 °C with an atmosphere of 5% CO<sub>2</sub>.

##### MTT assay

After homogenization, 5000 cells/well were seeded in 96 well microplates and kept in a desiccator under 5% CO<sub>2</sub> atmosphere. After two days of incubation, the cells were detected in an inverted microscope (lobomed) magnification (40x). Concentrations of 100 mg/mL and 200 mg/mL were prepared with DMSO for the (isolated compounds and Quercetin) as well as (methanol extract, hexane, ethyl acetate, butanol, and water fractions) respectively. The working concentration was prepared by adding 175 µL of media to 25 µL of the samples to obtain (25 mg/mL) and (12.5 mg/mL) for the (extract/fractions), and (compounds/Quercetin) respectively. The stock solution for the drug (Paclitaxel) was prepared with media to obtain 3 mg/ml. All the working concentration was prepared freshly as mentioned above, and filtered through 0.22 micron filter before the assay. Then 100 µL of the working concentration of samples were added to the well and mixed well with 100 µL of media, this volume was transferred from first to last well by serial dilution to obtain the desired concentration of the samples (12.5, 6.25, 3.125,

1.5625 mg/mL). The drug had a concentration of (1.5, 0.75, 0.375, 0.1875 mg/mL).

The cytotoxicity activity of samples on MCF-7 cells was determined by the MTT (3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide) assay using Horuchi *et al*, 1988 and Senthilraja and Kathiresan, 2015 methods with slight modification.<sup>10,11</sup> Cells (1 × 10<sup>5</sup>/well) were plated in 0.2 mL of medium/well in 96-well plates. The wells have complete media, samples, and 20 µL of MTT were added. The plates were incubated for 4 hrs in a 5% CO<sub>2</sub> incubator for cytotoxicity. After incubation for 48 h, 100 µL of DMSO (solubilizing reagent) was added to each well and mixed well by micropipette and left for 60 sec. The presence of viable cells was visualized by the development of purple color due to formation of the formazan crystals. The experiment was performed in triplicate for the concentration of each test sample. The absorbance was measured using TECAN® microplate reader at 595 nm and using only media as the blank. IC<sub>50</sub> values were expressed in mg/mL relative to the solvent control (DMSO), which was serially diluted with media and were determined graphically by plotting concentration of the sample in X-axis and cell inhibition in Y-axis. Paclitaxel from (*Taxus brevifolia*) is a widely used clinical anticancer drug and Quercetin (flavonoid) was used as the positive control.

$$\text{Percentage cell inhibition} = 100 - \left[ \frac{(A_t - A_b)}{(A_c - A_b)} \right] \times 100$$

A<sub>t</sub> = Absorbance value of test samples

A<sub>b</sub> = Absorbance value of blank

A<sub>c</sub> = Absorbance of DMSO

#### Statistical analysis

All experiments were performed in triplicate. The IC<sub>50</sub> values were presented as mean ± standard error of mean.

## Results and Discussion

Two (2) Phytosterol compounds that were isolated from the methanol extract of *F. paniculata* roots were sterol glucoside (**1**), and sterol (**2**). Compound **1** was isolated as a white, amorphous powder from the hexane fraction. It has a glucose group attached to the steroidal backbone at C-3 (Figure 1a). The mass spectral data gave a molecular formula C<sub>34</sub>H<sub>58</sub>O<sub>6</sub>, m/z 562.41[M-Na]<sup>+</sup> and base peak at m/z [M]<sup>+</sup> 585.41. The FTIR showed frequencies at 660.72, 723.19, 799.46, 1017.72, 1071.76, 1106.69, 1165.24, 1365.87, 1462.92, 1573.19, 1637.51, 2849.08, 2917.27, 2961, 3347.19 (cm<sup>-1</sup>)<sub>vmax</sub>. The absorption bands at 1017 cm<sup>-1</sup> supported its steroidal nature.<sup>13</sup> Band at 3347 cm<sup>-1</sup> was due to the presence of O–H stretching. The absorption bands at 2917 cm<sup>-1</sup> were indicative of the presence of C–H stretching and 1462 cm<sup>-1</sup> for C–H bending (alkane). <sup>1</sup>H-NMR (DMSO, 400 MHz) δ 0.67 (3H, d, J = 8.01 Hz), 0.73 – 0.87 (9H, m), 0.91 (3H, d, J = 6.39 Hz), 0.96 (3H, s), 0.98 – 1.07 (3H, m), 1.14 (2H, dd, J = 14.42, 8.38 Hz), 1.16 – 1.27 (1H, m), 1.29 (9H, s), 1.31 – 1.44 (1H, m), 1.45 – 1.56 (3H, m), 1.64 (1H, td, J = 7.01, 6.95, 4.97 Hz), 1.80 (3H, d, J = 12.94 Hz), 1.97 (2H, d, J = 12.53 Hz), 2.13 (1H, t, J = 11.98, 11.98 Hz), 2.33 – 2.42 (1H, m), 2.90 (1H, td, J = 8.44, 8.35, 4.72 Hz), 3.05 (2H, ddd, J = 13.58, 7.32, 3.53 Hz), 3.13 (1H, td, J = 9.14, 8.86, 4.88 Hz), 3.44 (2H, ddt, J = 23.91, 11.93, 6.51, 6.51 Hz), 3.65 (1H, ddd, J = 11.74, 5.83, 1.96 Hz), 4.22 (1H, d, J = 7.74 Hz), 4.42 (1H, t, J = 5.78, 5.78 Hz), 4.83 – 4.92 (3H, m), 5.33 (1H, d, J = 4.93 Hz). <sup>13</sup>C-NMR (101 MHz) δ 36.83 (C-1), 29.26 (C-2), 70.10 (C-3), 38.30 (C-4), 140.44 (C-5), 121.20 (C-6), 31.42 (C-7), 31.37 (C-8), 49.60 (C-9), 36.21 (C-10), 20.59 (C-11), 33.34 (C-12), 41.85 (C-13), 56.17 (C-14), 23.86 (C-15), 25.44 (C-16), 55.43 (C-17), 11.67 (C-18), 18.61 (C-19), 35.48 (C-20), 18.93 (C-21), 22.60 (C-22), 27.78 (C-23), 45.14 (C-24), 28.70 (C-25), 19.09 (C-26), 19.71 (C-27), 11.78 (C-28), 100.78 (C-1'), 73.46 (C-2'), 76.74 (C-3'), 76.77 (C-4'), 76.91 (C-5'), 61.09 (C-6'). The <sup>1</sup>H-NMR spectrum revealed the presence of fifty-eight (58) protons, olefinic proton resonated as a broad singlet at chemical shift (δ<sub>H</sub>) 5.33 ppm, which is indicative of the presence of a –C=C– in the

ring system. The peak at  $\delta_H$  4.42 ppm (1H), and 4.83 – 4.92 ppm (3H) correspond to the hydroxyl (OH) moieties of the glucose group. A number of multiplet signals between  $\delta_H$  0.73-0.87 ppm, 0.98-1.07 ppm, 1.45-1.56 ppm were indicative of different methyl and methylene protons in the structure of compound **1**.  $^{13}C$ -NMR spectrum revealed the presence of 34 carbons suggestive of a conjugated steroidal compound, this corresponding to six (6) methyls, eleven (11) methylenes, fourteen (14) methines, and three (3) quaternary carbons. In the Distortionless enhancement by polarization transfer (DEPT) NMR spectrum, eleven (11) methylenes were observed. The signals at 140.44 (C-5), 41.85 (C-13) and 36.21 (C-10) were assigned to three quaternary carbons. The signal at 70.10 ppm was for oxymethine carbon (C-3) (Table 1). The above spectra data is similar to the  $^1H$ - and  $^{13}C$ -NMR reported by Kolak *et al.*, 2005<sup>12</sup> and Khatun *et al.*, 2012<sup>13</sup> for Sitosterol glucoside, however, the peak for methylene ( $CH_2$  group) at C-28 was absent in the spectrum for Compound **1** (Figure 1a). Consequently, the structure of **1** was established as shown in figure 1 and was identified as Campesterol glucoside.

Compound **2** was isolated as white crystalline solid from the hexane fraction. The mass spectrum data gave a molecular formula  $C_{29}H_{50}O$ ,  $m/z$  397.38479 with molecular weight calculated as 414.38479  $g/mol[M+H_2O]$ . The FTIR showed frequencies at 800.01, 957.42, 1021.71, 1052.22, 1260.42, 1375.19, 1462.59, 2863.38, 2932.36, 2960.92, 3420 ( $cm^{-1}$ )  $\nu_{max}$ . The absorption band at  $3420\text{ cm}^{-1}$  was due to the presence of O–H stretching. The bands at  $2932\text{ cm}^{-1}$  were indicative of the presence of C–H stretching and  $1462\text{ cm}^{-1}$  for C–H bending (alkane). The absorption bands at  $1052\text{ cm}^{-1}$  supported its steroidal nature.  $^1H$ -NMR ( $CDCl_3$ , 400 MHz)  $\delta$  0.68 (2H, d,  $J = 7.25$  Hz), 0.74 – 0.90 (8H, m), 0.87 – 0.95 (2H, m), 0.91 – 1.06 (3H, m), 1.00 (2H, s), 1.11 (10H, s), 1.02 – 1.30 (5H, m), 1.26 – 1.50 (2H, m), 1.50 (3H, dt,  $J = 10.27, 3.92, 3.92$  Hz), 1.51 – 1.63 (2H, m), 1.67 (2H, dtd,  $J = 13.52, 6.84, 6.03, 4.08$  Hz), 1.83 (3H, ddd,  $J = 13.15, 6.96, 3.86$  Hz), 1.90 – 2.11 (2H, m), 2.16 – 2.34 (2H, m), 3.46 – 3.64 (1H, m), 5.35 (1H, t,  $J = 2.35, 2.35$  Hz).  $^{13}C$ -NMR (101 MHz)  $\delta$  37.08 (C-1), 31.48 (C-2), 71.63 (C-3), 42.12 (C-4), 140.58 (C-5), 121.54 (C-6), 31.73 (C-7), 31.70 (C-8), 49.96 (C-9), 36.33 (C-10), 20.91 (C-11), 39.60 (C-12), 42.15 (C-13), 55.89 (C-14), 24.13 (C-15), 28.07 (C-16), 56.59 (C-17), 11.68 (C-18), 18.61 (C-19), 35.97 (C-20), 18.86 (C-21), 33.77 (C-22), 25.91 (C-23), 45.66 (C-24), 28.98 (C-25), 19.22 (C-26), 19.64 (C-27), 22.90 (C-28), 11.81 (C-29). The  $^1H$ -NMR spectrum revealed the presence of fifty (50) protons and it has a hydroxyl group (OH) attached to the steroidal skeleton at C-3. The  $^1H$  spectrum showed a downfield intensity at  $\delta_H$  5.35 ppm (triplet), indicative of olefinic proton (H-6). The spectrum had a multiplet at  $\delta_H$  3.46-3.64 ppm indicative of an oxymethine proton (H-3). The  $^{13}C$  spectra revealed twenty-nine (29) carbons suggestive of a steroidal compound corresponding to six (6) methyls, eleven (11) methylenes, nine (9) methines, and three (3) quaternary carbons. In the Distortionless enhancement by polarization transfer NMR spectrum, eleven (11) methylenes were observed. The signals at C 140.58 (C-5), 42.15 (C-13) and 36.33 (C-10) were assigned to three quaternary carbons. The signal at C-71.63 ppm was for oxymethine carbon (C-3)<sup>13</sup> (Table 2).

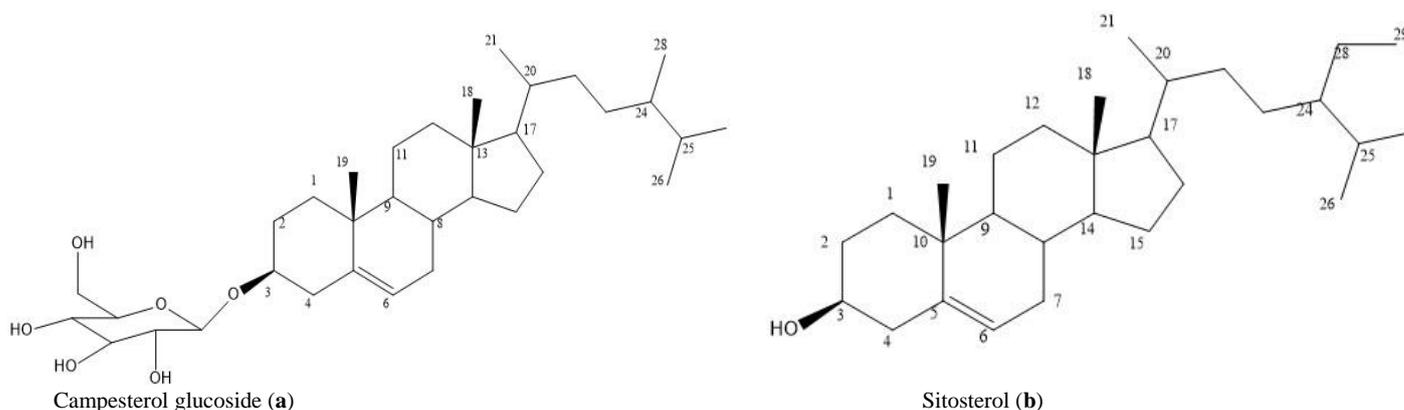
The above spectra data is similar to the  $^1H$  and  $^{13}C$  NMR reported by Sofodiya *et al.*, 2019<sup>9</sup> and Khatun *et al.*, 2012.<sup>13</sup> Hence, the structure of compound **2** was established as shown in figure 1b and was identified as **Sitosterol**. This is the first time these known compounds (Campesterol glucoside and Sitosterol) will be isolated from the roots of *Flabellaria paniculata*.

From the biological assay, the hexane fraction had the highest activity against MCF-7 breast cancer cells amongst the fractions tested, with  $IC_{50}$  0.97 mg/mL, hence further experiment was conducted on this fraction which led to the isolation of 2 compounds (Campesterol glucoside and Sitosterol). This may also suggest that much of the compounds in *F. paniculata* are in the non-polar form. The effect of sitosterol on MCF-7 breast cancer cells has been reported by some authors Chai *et al.*, 2008; Mariquit *et al.*, 2017; Nagla *et al.*, 2017.<sup>14,15,16</sup> However, when sitosterol with  $IC_{50}$  (1.79 mg/mL) and campesterol glucoside (1.18 mg/mL) was compared to the activity of the clinically used anticancer drug, paclitaxel (*Taxus brevifolia*) (0.07 mg/mL), it showed less activity against MCF-7 breast cancer cell lines. However, campesterol glucoside and sitosterol showed better activity than a well-known flavonoid, Quercetin (2.05 mg/mL). The reason for the better activity of campesterol glucoside than sitosterol against MCF-7 may be due the conjugated nature of the compound as a sterol glucoside. Although further *in vivo* studies are required to confirm the preclinical findings for campesterol glucoside. This is the first time the biological activity on MCF-7 cells of campesterol glucoside and extracts from *F. paniculata* will be reported.

**Table 1:** Cytotoxic test of extract, fractions, isolated compounds, with quercetin and paclitaxel as positive the control against MCF-7 breast cancer cell line

Compounds	$IC_{50} \pm SEM$ (mg/mL)
Methanol extract	2.73 $\pm$ 0.07
Hexane fraction	0.97 $\pm$ 0.05
Ethylacetate fraction	2.97 $\pm$ 0.19
Butanol fraction	6.22 $\pm$ 0.17
Water fraction	4.10 $\pm$ 0.13
Campesterol glucoside ( <b>1</b> )	1.18 $\pm$ 0.18
$\beta$ -sitosterol ( <b>2</b> )	1.79 $\pm$ 0.14
Quercetin	2.05 $\pm$ 0.16
Paclitaxel	0.07 $\pm$ 0.03

\*SEM: Standard error of mean



**Figure 1:** Molecular structure of compounds isolated from *F. paniculata* roots

**Table 1:** NMR spectra data of Campesterol glucoside

Position	DEPT	$\delta_C$ (ppm)	$\delta_H$ (ppm) (multiplicity, <i>J</i> in Hz)
1	CH <sub>2</sub>	36.83	1.80 (d, <i>J</i> = 12.94)
2	CH <sub>2</sub>	29.26	1.80 (d, <i>J</i> = 12.94), 1.29 (s)
3	CH	70.10	3.05 (ddd, <i>J</i> = 13.58, 7.32, 3.53)
4	CH <sub>2</sub>	38.30	2.13 (t, <i>J</i> = 11.98), 2.33 - 2.42 (m)
5	C	140.44	-
6	CH	120.20	5.33 (d, <i>J</i> = 4.93)
7	CH <sub>2</sub>	31.42	1.97 (d, <i>J</i> = 12.53)
8	CH	31.37	3.13 (td, <i>J</i> = 9.14, 8.86, 4.88),
9	CH	49.60	1.16 – 1.27 (m)
10	C	36.21	-
11	CH <sub>2</sub>	20.59	1.45 -1.56 (m)
12	CH <sub>2</sub>	33.34	1.31 -1.44 (m), 1.45 - 1.56 (m)
13	C	41.85	-
14	CH	56.17	0.98 -1.07 (m)
15	CH <sub>2</sub>	23.86	0.98 - 1.07 (m), 1.45 -1.56 (m)
16	CH <sub>2</sub>	25.44	1.14 (dd, <i>J</i> = 14.42, 8.38)
17	CH	55.43	0.98 -1.07 (m)
18	CH <sub>3</sub>	11.67	0.67 (d, <i>J</i> = 8.01)
19	CH <sub>3</sub>	18.61	0.73 - 0.87 (m)
20	CH	35.48	1.31 - 1.44 (m)
21	CH <sub>3</sub>	18.93	0.91 (d)
22	CH <sub>2</sub>	22.60	1.29 (s)
23	CH <sub>2</sub>	27.78	1.29 (s)
24	CH	45.14	1.29 (s)
25	CH	28.70	1.64 (td, <i>J</i> = 7.01, 6.65, 4.97)
26	CH <sub>3</sub>	19.09	0.96 (s)
27	CH <sub>3</sub>	19.71	0.73 - 0.87 (m)
28	CH <sub>3</sub>	11.78	0.73 - 0.87 (m)
Glucoside			
1'	CH	100.78	4.22(d, <i>J</i> = 7.74)
2'	CH	73.46	2.90 (td, <i>J</i> = 8.44, 8.35, 4.72)
3'	CH	76.74	3.05 (ddd, <i>J</i> = 13.58, 7.32, 3.53)
4'	CH	76.77	3.65 (ddd, <i>J</i> = 11.74, 5.83, 1.96)
5'	CH	76.91	2.90 (td, <i>J</i> = 8.44, 8.35, 4.72)
6'	CH <sub>2</sub>	61.09	3.44 (ddt, <i>J</i> = 11.93, 6.51)

**Table 2:** NMR spectra data of sitosterol

Position	DEPT	$\delta_C$ (ppm)	$\delta_H$ (ppm) (multiplicity, <i>J</i> in Hz)
1	CH <sub>2</sub>	37.08	1.02 – 1.30 (m)
2	CH <sub>2</sub>	31.48	1.90 – 2.11 (m)
3	CH	71.63	3.46 – 3.64 (m)
4	CH <sub>2</sub>	42.12	2.16 – 2.34 (m)
5	C	140.58	-
6	CH	121.54	5.35 (t, <i>J</i> = 2.35, 2.35)
7	CH <sub>2</sub>	31.73	1.83 (ddd, <i>J</i> = 13.15, 6.96, 3.86)
8	CH	31.70	1.50 (dt, <i>J</i> = 10.27, 3.92, 3.92)
9	CH	49.96	0.74 – 0.90 (m),
10	C	36.33	-
11	CH <sub>2</sub>	20.91	1.50 (dt, <i>J</i> = 10.27, 3.92, 3.92)
12	CH <sub>2</sub>	39.60	1.11 (s)
13	C	42.15	-
14	CH	55.89	0.87 – 0.95 (m)
15	CH <sub>2</sub>	24.13	1.11 (s)
16	CH <sub>2</sub>	28.07	1.51 – 1.63 (m)
17	CH	56.59	0.87 – 0.95 (m)
18	CH <sub>3</sub>	11.68	0.74 – 0.90 (m)
19	CH <sub>3</sub>	18.61	0.74 – 0.90 (m)
20	CH	35.97	1.02 – 1.30 (m)
21	CH <sub>3</sub>	18.86	1.11 (s),
22	CH <sub>2</sub>	33.77	1.26 – 1.50 (m)
23	CH <sub>2</sub>	25.91	1.00 (s),
24	CH	45.66	0.74 – 0.90 (m)
25	CH	28.98	1.83 (ddd, <i>J</i> = 13.15, 6.96, 3.86)
26	CH <sub>3</sub>	19.22	1.11 (s),
27	CH <sub>3</sub>	19.64	0.91 – 1.06 (m)
28	CH <sub>2</sub>	22.90	1.67 (dtd, <i>J</i> = 13.52, 6.84, 6.03, 4.08)
29	CH <sub>3</sub>	11.81	0.68 (d, <i>J</i> = 7.25)

## Conclusion

The study revealed that the roots of *F. paniculata* have inhibitory effect on MCF-7 breast cancer cell. The hexane fraction was the most active among the fractions, while campesterol glucoside showed a better activity than sitosterol. However, the standard drug, paclitaxel was the most active comparatively. This activity may account for the traditional use of this plant in the treatment of breast cancer in southwest Nigeria.

## Conflict of interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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