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**Resveratrol derivatives from *Commiphora africana* (A. Rich.) Endl. display cytotoxicity and selectivity against several human cancer cell lines**

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## Abstract

*Commiphora africana* (A. Rich.) Endl. (Burseraeae) is a medicinal plant widely used in Nigerian ethnomedicine. The *in vitro* cytotoxicity of the stem bark extract of *C. africana* and isolated cytotoxic compounds was investigated. Three resveratrol derivatives; (*E*)-resveratrol 3-*O*-rutinoside (**1**), 5-methoxy-(*E*)-resveratrol 3-*O*-rutinoside (**2**) and pinostilbene (**3**), together with 3-hydroxy-5-methoxybenzoic acid (**4**) were isolated from the methanol fraction of *C. africana*. Their structures were determined by extensive analysis of their HREIMS and NMR spectra. The cytotoxicity of the isolated compounds against four human carcinoma cells were determined using the MTT assay. Compound **1** displayed the highest antiproliferative effect on the cell lines, with IC<sub>50</sub> values of 16.80, 21.74, 17.89 and 17.44 μM, against MCF7, A549, PC3 and HepG2 human cancer cell lines, respectively. In addition, compounds **1-3** showed low toxicity against normal human prostate cell line, with selectivity indices greater than five across the carcinoma cells, indicating the compounds possess potential in the development of low-toxicity chemotherapeutic agents. These results support the traditional use of this plant in the treatment of cancer.

Keywords: Burseraeae, *Commiphora africana*, cytotoxicity, MTT assay, resveratrol derivatives

## 1. INTRODUCTION

Cancer continues to pose a major public health concern worldwide, with approximately 8.7 million cancer related deaths and 17.5 new million cases in 2015 (Fitzmaurice et al., 2017). After cardiovascular diseases, like ischaemic heart disease, cancer is the second leading cause of death globally with lung cancer alone responsible for 1.7 million death worldwide in 2015 (WHO, 2017). The common cancer types in decreasing order of mortality in 2015 are: lung cancer (1.69 million deaths), hepatic (788 000 deaths), colorectal (774 000 deaths), stomach (754 000 deaths) and breast (571 000 deaths) (WHO, 2017). Physical carcinogens, chemical carcinogens and biological carcinogens, are external agents that have been extensively linked to cancer. However, the single most important risk factor for cancer is tobacco use, which causes one out of five global cancer deaths and nearly 70% of global lung cancer deaths (Torre et al., 2015; Cohen et al., 2017). Despite the various treatment modalities available, the problem of poor selectivity and severe side effects of the available anticancer drugs have limited cancer chemotherapy success. This situation is complicated by the emergence and spread of multidrug resistance (MDR) in cancer cells, which occurs through several mechanisms, such as the p53 tumour suppressor genes mutations (Li et al., 2014), over-expression of ABC transporters (Kalapos-Kovács et al., 2015) and the epidermal growth factor receptor (Safe and Kasiappan, 2016). These factors have necessitated the need for the search and development of safer and more effective chemotherapeutic agents.

Natural products have been used from earliest times for the treatment of many ailments. Approximately 70% of the drugs used today are models of natural products. In addition, secondary metabolites derived from natural products are the most successful source of potential drug leads (Rey-Ladino et al., 2011; Newman and Cragg, 2016). Over the past five decades, many medicinal plants have been screened for cytotoxicity. Although Africa hosts 57,704 species of the world's flora and Africans utilise about 5,000 plants for medicinal purposes (Tariq et al., 2017), the discovery of anticancer drug leads from African medicinal plants has not been fully realised as plants from Chinese herbal medicine. For instance, camptothecin, a cytotoxic alkaloid that inhibits DNA topoisomerase I, was isolated from the bark of *Camptotheca acuminata*, a plant used for the treatment of cancer in traditional Chinese medicine. Homoharringtonine, from *Cephalotaxus harringtonia* var. *drupacea*, is a cytotoxic agent in clinical use discovered from traditional Chinese medicine (Newman and Cragg, 2016). Recently, some African countries have begun to make a commitment to discover bioactive drug

leads from indigenous medicinal plants. The recent screening of 7500 species of medicinal plants from South Africa for anticancer activity (Fouche et al., 2008) and various investigative work by scientists in Nigeria (Ashidi et al., 2010; Engel et al., 2011; Fadeyi et al., 2013; Ogbole et al., 2017; Segun et al., 2018a; Segun et al., 2018b) attest to this claim.

In the present report, the continuous search of potential antiproliferative agents from the Nigerian flora was extended to *Commiphora africana* (A. Rich.) Endl. (Burseraceae). This plant is a tree of not more than 5 m high found in dry sites and savannah forest of Africa. The tree has a short trunk with a creeping root system that spreads several metres around the tree (Aliyu et al., 2007). The leaves of *C. africana* are used traditionally to treat menstrual pains, painful swellings and wounds (Adebayo et al., 2006). Typical of the genus *Commiphora*, the tree produces a resinous gum which is used medicinally to seal and disinfect wounds. The fruits are used for the treatment of abdominal cramps and typhoid infection (Ezekiel et al., 2010). Previous biological investigations revealed that the plant possesses anti-lipidemic, anti-inflammatory, anti-oxidative, anthelmintic and antimicrobial activities (Compaoré et al., 2016; Gbolade and Adeyemi, 2008; Adebayo et al., 2006). Aliyu and co-workers reported that the ethanol leaf extract of *C. africana* may enhance liver and kidney function at low doses, but may elicit toxic effects on these organs at high concentration (Aliyu et al., 2007). The hydro-ethanolic extract of the stem-bark of the plant was shown to demonstrate anti-convulsive activity and possess the ability to regulate sleep disorders in mice (Ezekiel et al., 2010). Phellamurin 1, a dihydroflavonol glucoside isolated from the dichloromethane-methanol extract of *C. africana* mediated  $\text{Cu}^{2+}$ -dependent relaxation of supercoiled plasmid DNA (Ma et al., 2005). Recently, we conducted an ethnobotanical study of medicinal plants used for the management of cancer in local communities of south western Nigeria. Our findings revealed that *C. africana* was one of the most frequently used medicinal plants for the treatment of cancer. Other recent ethnobotanical surveys from the northern part of Nigeria also documented the use of the stem bark of the plant for the treatment of cancer (Ngulde et al., 2015; Dafam et al., 2016). Therefore, the present study was designed to investigate the cytotoxicity of the methanol (MeOH) extract of the stem bark of *C. africana*, its solid-phase extraction (SPE) and fractions and isolated compounds against four human carcinoma cell lines: breast (MCF7), liver (HepG2), lung (A549), prostate (PC3) and the immortalised human prostatic epithelial cells (PNT-2) using the *in vitro* 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) viability assay.

## **2 MATERIALS AND METHODS**

### **2.1 General**

UV spectra was obtained with an Analytik Jena UV Visible spectrophotometer (Germany) in MeOH. Infra-red spectra analysis were carried out on a Perkin Elmer FTIR Spectrum BX spectrometer. <sup>1</sup>H- and <sup>13</sup>C- NMR spectra were recorded at 600 MHz and 150 MHz, respectively, on a Bruker Avance III Spectrometer. Quaternary, methine, methylene and methyl carbons were differentiated by DEPT experiments. COSY experiments were used to determine <sup>1</sup>H homonuclear connectivities. HSQC experiments were used to determine <sup>1</sup>H-<sup>13</sup>C one-bond connectivities experiment while HMBC experiments were used to determine two and three <sup>1</sup>H-<sup>13</sup>C bond connectivities. Chemical shifts were reported in  $\delta$  (ppm) using deuterated methanol as the solvent and internal standard and the coupling constants (*J*) were measured in Hertz (Hz). Mass spectrometric (HREIMS) data were generated using the Thermo Scientific LTQ Orbitrap XL mass spectrometer at the National Mass Spectrometry Facility (Swansea, England). Reversed-phase preparative (RP-Prep) HPLC analysis was performed on an Agilent 1260 infinity HPLC System (Agilent Technologies), equipped with an Agilent 1260 preparative binary pumps, an Agilent 1260 HiP degasser, an Agilent column chamber, an Agilent 1260 diode array detector and an Ace-5 C18 column (150 × 21.2 mm, 5  $\mu$ m particle size, Hichrom Ltd. HPLC grade solvents were purchased from Fischer Scientific (Loughborough, UK). Absorbance measurement for the MTT assay were taken on a CLARIO star microplate reader (BMG labtech, Germany).

### **2.2 Cell culture materials**

Human adenocarcinoma cell lines: breast (MCF7), liver (HepG2), lung (A549) and prostate (PC3), were purchased from the American Type Culture Collection (ATCC), (LGC Standards, Middlesex, UK) and the normal human immortalised prostate cell line (PNT2) was obtained from the European Collection of Authenticated Cell Cultures (ECACC), (Public Health England, Salisbury, UK). Cell culture materials including Dulbecco's Modified Eagle Medium (DMEM), foetal bovine serum (FBS) and trypsin were purchased from Biosera (Nouaille, France). Chemicals including 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium (MTT), vinblastine, trypan blue, phosphate buffered-saline (PBS) tablets and penicillin-streptomycin antibiotics suspension were purchased from Sigma-Aldrich (Dorset, UK).

### **2.3 Plant materials**

*Commiphora africana* stem bark was collected from Jebba (9°07'23.5"N 4°49'35.8"E), Niger State, Nigeria, in August 2016 and identified by Mr. Bolu Ajayi, herbarium botanist, Department of Plant Biology, University of Ilorin, Nigeria. The herbarium specimen for this collection (voucher number: UILH/0001/1248) has been deposited and retained in the University of Ilorin Herbarium.

## 2.4 Extraction and isolation of active compounds

The powdered dried bark of *C. africana* (170 g each) was extracted using a Soxhlet apparatus with 800 mL each of *n*-hexane, dichloromethane and methanol (MeOH). To ensure exhaustive extraction, ten cycles were allowed for each extraction and the heating mantle was kept constant at 50 °C for all extractions. Filtered extracts were concentrated and dried using a rotary evaporator at 40 °C and stored in the refrigerator prior to use. A Strata C-18 cartridge (35µm; 70Å; 20 g) was washed with MeOH (50 mL) followed by equilibration with HPLC grade water (100 mL). The dried MeOH extract (2 g) was dissolved in 10 mL of HPLC grade water and loaded on to the cartridge (Basar et al., 2015). The cartridge was eluted with a binary gradient solvent system to obtain four fractions: 20, 50, 80 and 100% MeOH in water (250 mL each), coded respectively as CAF1, CAF2, CAF3 and CAF4. All four fractions were evaporated to dryness and stored in the refrigerator prior to cytotoxic screening. CAF2 and CAF3, which were the most active against the cancer cell lines under investigation, were further purified as described below. CAF2 (311 mg) was purified on reversed phase preparative (RP-Prep) HPLC (mobile phase H<sub>2</sub>O/MeOH containing 0.1% trifluoroacetic acid (TFA); 0-30 min: 20-80% MeOH, 30-35 min: 80-100% MeOH, 35-40 min: 100% MeOH, 40-45 min: 20% MeOH; flow rate, 10 mL/min; UV detection at 220, 254, and 320 nm) to afford 3-hydroxy-5-methoxybenzoic acid (**4**, 9.9 mg; *t<sub>R</sub>*=11.2 min), resveratrol 3-*O*-rutinoside (**1**, 10.8 mg; *t<sub>R</sub>*=12.6 min), and 5-methoxy-(*E*)-resveratrol 3-*O*-rutinoside (**2**, 22.6 mg; *t<sub>R</sub>*=22.4 min). CAF3 (72 mg) was purified on RP-Prep HPLC (mobile phase H<sub>2</sub>O/MeOH containing 0.1% trifluoroacetic acid (TFA); 0-30 min: 30-100% MeOH, 30-35 min: 100% MeOH, 35-40 min: 30% MeOH; flow rate, 10 mL/min; UV detection at 220, 254, and 320 nm) to afford pinostilbene (**3**, 11.3 mg; *t<sub>R</sub>*=16.8 min).

## 2.5 Cytotoxicity studies

### 2.5.1 Cell culture

All cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% foetal bovine serum (FBS) and 1% penicillin-streptomycin antibiotics suspension, in

a humidified atmosphere of 95% air and 5% CO<sub>2</sub> at 37 °C. Cells were washed with phosphate buffered saline and harvested by trypsinisation. Cell count was made using an automated haemocytometer (C-Chip NanoEnTek, USA). Morphological changes to cells, as a result of the effects of test compounds, were observed through an inverted microscope (40x and 100x, Olympus CKX41, UK).

### **2.5.2 Preparation of extracts and compounds**

Plant extracts and fractions were dissolved in 10% dimethyl sulfoxide (DMSO), to give a stock solution (SS) of 100 mg/mL. Vortex agitation was used to ensure the uniformity of the solution prior to use. From the SS, serial dilutions were made in the cell culture media to obtain working concentrations of 100, 20, 4, 0.8 and 0.16 µg/mL. For the pure compounds, a SS of 1 M was prepared, thereafter, serial dilutions were made to give working concentrations of 100, 50, 25, 12.5 and 6.25 µM for treatment with MCF7, HepG2, A549 and PC3, and working concentrations of 800, 400, 200, 100 and 50 µM for treatment with PNT2. For vinblastine (positive control), a SS of 10 µM was prepared, thereafter, serial dilutions were made to give working concentrations of 100, 50, 25, 12.5, 6.25 nM. Dilutions of the SS were made in cell culture media, with the final concentration of DMSO not exceeding 0.1%, which had previously been found not to be cytotoxic (Basar et al., 2015).

### **2.5.3 Colorimetric MTT *in vitro* assay**

Cytotoxicity testing was carried out using the MTT assay, which determines the number of viable cells in culture (Gerlier and Thomasset, 1986). After repeated initial experiments, the cell densities for optimal growth were found to be  $2 \times 10^4$ ,  $4 \times 10^4$ ,  $1.2 \times 10^4$ ,  $2 \times 10^4$  and  $4 \times 10^4$  cells/well for MCF7, HepG2, A549, PC3 and PNT2, respectively. The exponential phases of the cell lines were seeded into flat-bottomed, 24-well plate (Corning Costar® TC-treated, Merck, UK) and incubated at 37°C/5% CO<sub>2</sub> for 24 h, to allow the cells to adhere to the surface of the plate. After reaching about 90% confluency at 24 h, the old medium was removed by vacuum suctioning, and the cells treated with varying concentrations (1 mL each) of the extract(s), fraction(s) and compound(s), and incubated for 72 h. The first five wells of the 24-well plate (A1-A5) contained the various concentrations of the extract/fraction/compound to be tested while the last well (A6) contained only 1 mL of the DMSO-growth medium (negative control). The experiment was conducted in quadruplicate for each test dilutions, so that wells A1-D1, A2-D2, A3-D3, A4-D4, A5-D5 contained different concentrations of the extract/fraction/compound. At the expiration of the incubation period, the MTT assay was



performed to measure the cell viability. The protocol described earlier in the literature was adopted with slight modifications (Mosmann, 1983). Briefly, the old medium was removed from each well by vacuum suctioning and replaced with 1 mL of 500 µg/mL MTT dye in PBS. The plates were incubated for 2 h at 37°C/5% CO<sub>2</sub> to allow the production of formazan crystals. After incubation, the medium was removed by vacuum suctioning and 500 µL of propanol was added under light protection to dissolve the dark blue formazan crystals. The plates were placed on a shaker for 10 min to enhance the dissolution of preformed formazan crystals. The dilutions in each 24-well plate were transferred into a 96 well plate so that the solution in A1 (24-well) was transferred to A1-A4 (96-well), B1 (24-well) was transferred to B1-B4 (96-well) and so on. The absorbance of the solution was measured at 570 nm on a Clario Star microplate reader. The average optical density (OD) obtained from the negative control wells was set arbitrarily at 100% and the average OD of treatment wells was calculated relative to the negative control. In addition, the same process was carried out for vinblastine (positive control) using the concentration range mentioned above.

To obtain the selectivity indices (SI) of the isolated compounds, the normal PNT2 cell line was used. The SI value was calculated as the ratio of the IC<sub>50</sub> values of the compounds on PNT2 cells relative to those in the cancer cell lines.

Two sets of 24 well plates were used to determine whether the antiproliferative activities of the compounds **1-3** were cytostatic or cytotoxic. The first plate measured the cell growth inhibition after 72 h treatment (exposure assay), while the second plate determined the cell growth inhibition after a further 72 h period, following replacement of the old medium containing the test compound with fresh growth medium alone (recovery assay) (Ashidi et al., 2010). The antiproliferative effect is cytostatic when the IC<sub>50</sub> value of the recovery assay is higher than that of the exposure assay, whereas the effect is cytotoxic when the IC<sub>50</sub> value is similar in both recovery and exposure assay (Wong et al., 2011).

## 2.6 Statistical analysis

The results obtained were expressed as mean ± standard error of means (SEM). The experiments were conducted in quadruplicate on three different occasions. The cell viability was calculated as:

$$\% \text{ Inhibition} = \frac{\text{Mean OD}_{\text{control}} - \text{Mean OD}_{\text{treated}}}{\text{Mean OD}_{\text{Control}}} \times 100$$

The statistical analysis was done using GraphPad (Version 5.01, GraphPad Prism Software Inc., San Diego, CA.). One-way analysis of variance (ANOVA) followed by Tukey's test (each treatment compared to control) were used to test for the statistical differences between the groups. Differences between means were considered statistically significant when  $P < 0.05$ . IC<sub>50</sub> values were obtained from graphs plotted using non-linear regression equation log (inhibitor) versus response - variable slope.

### 3. RESULTS AND DISCUSSION

#### 3.1 Structure determination

As the MeOH fraction of *C. africana* displayed significant cytotoxicity against several cell lines, it was fractionated into four parts using the SPE. The isolated compounds from the active CAF2 and CAF3 were identified as (*E*)-resveratrol 3-*O*-rutinoside (**1**), 5-methoxy-(*E*)-resveratrol 3-*O*-rutinoside (**2**), pinostilbene (**3**) and 3-hydroxy-5-methoxybenzoic acid (**4**) (Figure 1) by direct comparison of their spectroscopic data (<sup>1</sup>H NMR, <sup>13</sup>C NMR, <sup>13</sup>C DEPTQ, <sup>1</sup>H-<sup>13</sup>C HSQC, <sup>1</sup>H-<sup>13</sup>C HMBC and <sup>1</sup>H-<sup>1</sup>H NOESY) with those in the literature (Wanjala and Majinda, 2001; Tyukavkina et al., 1972).

(*E*)-Resveratrol 3-*O*-rutinoside (**1**): amorphous, white powder; UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 206 (1.38), 302 (0.44) nm; IR (KBr)  $\nu_{\max}$  3303, 1587, 1512, 1432, 1040 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data, see Table S1 (Supporting information); negative HRESIMS  $m/z$  535.1820 [M-H]<sup>-</sup> (calcd for C<sub>26</sub>H<sub>31</sub>O<sub>12</sub>, 535.1821).

5-methoxy-(*E*)-resveratrol 3-*O*-rutinoside (**2**): amorphous, white powder; UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 218 (0.68), 308 (0.82), 322 (0.82) nm; IR (KBr)  $\nu_{\max}$  3304, 1587, 1512, 1433, 1040 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data, see Table S1 (Supporting information); positive HRESIMS  $m/z$  551.2130 [M+H]<sup>+</sup> (calcd for C<sub>27</sub>H<sub>35</sub>O<sub>12</sub>, 551.2130).

Pinostilbene (**3**): amorphous, white powder; UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 228 (1.02), 284 (0.53) nm; IR (KBr)  $\nu_{\max}$  3303, 1587, 1512, 1432, 1040 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data, see Table S1 (Supporting information); positive HRESIMS  $m/z$  243.1024 [M+H]<sup>+</sup> (calcd for C<sub>15</sub>H<sub>14</sub>O<sub>3</sub>, 243.1021).

3-Hydroxy-5-methoxybenzoic acid (**4**): amorphous, white powder; <sup>1</sup>H-NMR (600 MHz, CD<sub>3</sub>OD)  $\delta$ : 7.06 (2H, d, J=2.3 Hz, H-2, H-6), 6.58 (1H, d, J=2.3 Hz, H-4) and 54.4 (3H, s, 5-OCH<sub>3</sub>); <sup>13</sup>C-NMR (150 MHz, CD<sub>3</sub>OD)  $\delta$ : 132.4 (C-1), 105.7 (C-2), 158.4 (C-3), 105.6 (C-4), 160.9 (C-5), 108.9 (C-6) and 168.4 (C-7).

#### 3.2 Cytotoxicity assay

Soxhlet extraction of the stem bark of *C. africana* (290 g) led – after evaporation under vacuum – to *n*-hexane extract (4.14 g; 1.3% yield), DCM extract (1.36 g; 0.5% yield) and MeOH extract (16.5 g; 5.7% yield). These extracts were tested against MCF7 (breast), HepG2 (liver), A549 (lung) and PC3 (prostate) carcinoma cells according to the MTT assay and the cytotoxicity result is reported in Table 1. The cell lines used in this study were selected because of high incidence rate for these cancers, and the need to develop effective and safe medications for treatment. For example, the breast cancer is the most common form of cancer in female, while the prostate cancer is the most diagnosed cancer type in male. Furthermore, lung cancer was the most common cause of cancer deaths globally, accounting for 18.2% (1.7 million deaths) of the total cancer mortality in 2015 (Fitzmaurice et. al., 2017). The *n*-hexane extract showed no antiproliferative activity at the highest concentration used in this study, while the DCM extract displayed cytotoxicity with IC<sub>50</sub> values of 62.63, 61.92, 63.88 and 68.21 µg/mL against MCF7, A549, PC3 and HepG2, respectively. However, the MeOH fraction showed the highest cytotoxicity amongst the extracts, with IC<sub>50</sub> values of 12.14, 12.28, 14.95 and 16.15 µg/mL against MCF7, A549, PC3 and HepG2, respectively. As the cytotoxicity displayed by the MeOH extract of *C. africana* met the specification of the National Cancer Institute (NCI), which requires a crude plant extract to display IC<sub>50</sub> value < 30 µg/mL before it could be considered as active (Raj Kapoor et al., 2007), it was selected for fractionation by the solid phase extraction (SPE) method. The screening of the SPE fractions on the cancer cells using the MTT assay showed that, CAF2 and CAF3 displayed the highest cytotoxicity across the panel of carcinoma cells (Table 1). Purification of CAF2 and CAF3 using reversed-phase preparative HPLC analysis led to the isolation of four known compounds identified as (*E*)-resveratrol 3-*O*-rutinoside (**1**), 5-methoxy-(*E*)-resveratrol 3-*O*-rutinoside (**2**), pinostilbene (**3**) and 3-hydroxy-5-methoxybenzoic acid (**4**). With the exception of 3-hydroxy-5-methoxybenzoic acid (**4**) which showed no cytotoxicity on the four cancer cell lines at concentration below 200 µM, the test compounds displayed concentration-dependent cytotoxicity across the four human carcinoma cells (Figure 2). As the test compound concentration increased, there was a resultant loss of normal elongated shape of viable cells, confluence of cells reduced, remaining viable cells shrunk to smaller round cells, ultimately leading to cell death. The obtained IC<sub>50</sub> values ranged from 16.80 µM (MCF7 cells) to 21.74 µM (A549 cells) for (*E*)-resveratrol 3-*O*-rutinoside; from 25.50 µM (HepG2 cells) to 31.78 µM (PC3 cells) for 5-methoxy-(*E*)-resveratrol 3-*O*-rutinoside; from 29.36 µM (MCF7 cells) to 41.56 µM (A549 cells) for pinostilbene; and from 21 nM (HepG2) to 37 nM (A549) for vinblastine (Table 1). The obtained result indicates that, pinostilbene, which lacked the sugar moiety present in 5-

methoxy-(*E*)-resveratrol 3-*O*-rutinoside, had a less antiproliferative activity across the carcinoma cell lines.

In order to gain an insight into the mechanism of the cytotoxicity displayed by the isolated compounds, it was necessary to determine whether the antiproliferative effect were due to cytostatic effects or cytotoxic effects. It was observed that with the exception of the HepG2 cells, compounds **1** – **3** exhibited cytotoxic effects on the carcinoma cells, as the IC<sub>50</sub> values obtained were higher in the exposure assay compared to the recovery assay (Figure 3). It could be said that cytostatic effects were responsible for the cytotoxic effects of the test compounds in the HepG2 cells, as the compounds displayed higher IC<sub>50</sub> value in the recovery assay (27.24, 34.4 and 55.04 μM for (*E*)-resveratrol 3-*O*-rutinoside, 5-methoxy-(*E*)-resveratrol 3-*O*-rutinoside and pinostilbene, respectively), in comparison to the exposure assay (17.44, 25.5 and 35.05 μM for (*E*)-resveratrol 3-*O*-rutinoside, 5-methoxy-(*E*)-resveratrol 3-*O*-rutinoside and pinostilbene, respectively).

The selectivity indices (SI) of compounds **1-3** is shown in Table 2. The SI is calculated by comparing the IC<sub>50</sub> value on the carcinoma cell line to the IC<sub>50</sub> value in the normal human cell line. The normal immortalised human prostate cell line (PNT2) was used as the normal human cell and vinblastine was used as the positive control. Selectivity index value greater than one suggests that the compounds are less toxic to the normal cell compared to the cancer cells, and compounds that showed high SI value could be assumed to offer potential safer therapy. Interestingly, (*E*)-resveratrol 3-*O*-rutinoside which was the most active of the isolated compounds, also displayed the highest SI across the carcinoma cells, with SI ranging from 11.3 (A549 cells) to 14.7 (MCF7 cells). The other compounds also displayed SI value higher than that obtained for vinblastine, with values varying between 6.2 (PC3 cells) to 7.7 (HepG2 cells) for 5-methoxy-(*E*)-resveratrol 3-*O*-rutinoside and between 5.4 (A549 cells) to 7.7 (MCF7 cells) for pinostilbene. Vinblastine, the positive control, had a SI value ranging from 1.00 to 1.76 across the cancer cell lines, suggesting that the drug cannot sufficiently differentiate between normal and cancer cells, a result in agreement with a previous study (Ashidi et al., 2010). It could be inferred from the selectivity indices, that the isolated compounds, especially (*E*)-resveratrol 3-*O*-rutinoside, have potential in the development of low-toxicity chemotherapeutic agents.

Resveratrol derivatives are an important class of plant polyphenols, with promising diverse activities including antitumor, anti-inflammatory, antioxidant, antidiabetic and acetylcholinesterase inhibitory effects (Shen et al., 2009). While this is the first report on the

occurrence of the resveratrol derivatives (**1-3**) in the genus *Commiphora*, other genera of the family Burseraceae have been reported to produce some stilbene glycosides (Atta-ur-Rahman et al., 2005; Hussain et al., 2009). In addition, this is the first report on the cytotoxicity of (*E*)-resveratrol 3-*O*-rutinoside on human cancer cells. 5-Methoxy-(*E*)-resveratrol 3-*O*-rutinoside has been reported to display significant inhibition of phosphodiesterase I and xanthine oxidase (Atta-ur-Rahman et al., 2005), but demonstrated no lethality on brine shrimp larvae at concentrations of up to 1000 ppm (Wanjala and Majinda, 2001). An earlier study reported that 5-methoxy-(*E*)-resveratrol 3-*O*-rutinoside regulated angiogenesis by decreasing the activity of pro-angiogenic FGF-2 and also inhibited *in vivo* angiogenesis in the chick chorioallantoic membrane (CAM) assay (Hussain et al., 2009). Recent research showed that pinostilbene significantly inhibited the proliferation of human colon cancer cells (HCT116 and HT29), causing cell cycle arrest at the S phase, leading to the induction of apoptosis (Sun et al., 2016). Another study revealed that pinostilbene displayed *in vitro* cytotoxicity with IC<sub>50</sub> value of 78.13 μM against NCI-H187 cell line (Lertnitikul et al., 2016). Pinostilbene was shown to possess high antioxidant capacity displaying a value of 5.01 Trolox equivalents/μM in an oxygen radical absorbance capacity (ORAC) assay (Uesugi et al., 2017). The antioxidant capacity could have been contributed to the cytotoxicity of pinostilbene, reported in the present study.

To the best of our knowledge, this is the first report on the cytotoxicity of the MeOH extract of the stem bark of *C. africana*, and its SPE fraction, against human carcinoma cells. Previous studies have shown that members of the *Commiphora* genus possess compounds with antiproliferative potential. Stigmasta-5,22*E*-diene-3β,7α,11α-triol, a stigmastane-type steroid, isolated from the resinous exudates of *C. mukul* displayed inhibitory effects with IC<sub>50</sub> values of 5.21, 9.04, 10.94 and 16.56 μM, against K562, MCF7, PC3 and DU145 human cancer cell lines, respectively (Shen et al., 2012). Similarly, erlangerin A to D, lignans isolated from the resins of *C. erlangeriana* were shown to display cell growth inhibitory activity on two human (HeLa and EAhy926) and two murine (L929 and RAW 264.7) cell lines (Habtemariam, 2003).

## CONCLUSION

In this study, we report the antiproliferative potentials of the extracts and isolated compounds of *C. africana* on four human carcinoma cells. These results, validate the folklore use of *C. africana* in the treatment of cancer.

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## **CONFLICT OF INTEREST**

The authors have declared that there is no conflict of interest.

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Table 1. Antiproliferative Effects of the *n*-hexane, DCM, MeOH Extract, MEOH Fractions and Pure Isolates from the Stem Bark of *Commiphora africana* against Four Human Cancer Cell Lines<sup>a</sup>

Frct/ Cmpd	IC <sub>50</sub> (μg/mL) <sup>b</sup>				
	MCF7	A549	PC3	HepG2	PNT2
<i>n</i> -hexane	> 100	> 100	> 100	> 100	ND
DCM	62.63 ± 1.12	61.92 ± 1.19	63.88 ± 2.12	68.21 ± 1.03	ND
MeOH	12.14 ± 0.17	12.28 ± 1.03	14.95 ± 0.04	16.15 ± 0.07	ND
CAF1	22.81 ± 0.12	27.52 ± 2.87	25.39 ± 1.12	26.85 ± 0.22	ND
CAF2	10.15 ± 0.87	10.39 ± 0.16	9.77 ± 1.67	11.34 ± 0.25	ND
CAF3	12.67 ± 0.96	10.05 ± 0.76	11.12 ± 1.10	12.49 ± 0.46	ND
CAF4	24.41 ± 0.21	23.70 ± 1.28	27.10 ± 1.12	25.86 ± 0.23	ND
1	16.80 ± 0.51	21.74 ± 0.71	17.89 ± 0.77	17.44 ± 0.44	246.80 ± 4.03
2	27.54 ± 0.77	25.95 ± 0.87	31.78 ± 0.61	25.50 ± 0.96	196.31 ± 5.01
3	29.36 ± 0.12	41.56 ± 0.91	38.44 ± 0.77	33.05 ± 0.21	225.30 ± 3.42
4	>200	>200	>200	>200	>200
Vinblastine	0.023 ± 0.01	0.037 ± 0.01	0.028 ± 0.01	0.021 ± 0.01	0.057 ± 0.01

<sup>a</sup>MCF7, human breast adenocarcinoma; A549, human lung carcinoma; PC3, human prostate adenocarcinoma; HepG2, human hepatocellular carcinoma. <sup>b</sup>MTT method, with the cells incubated with the fractions for 72 h (means ± SEM, n = 3); Frct: fraction; Cmpd: compound; ND, not determined

Table 2. The Selectivity Indices (SI) of **1-3** and Vinblastine (VBN)

Compound	MCF7	A549	PC3	HepG2
1	14.69	11.35	13.79	14.15
2	7.12	7.56	6.17	7.69
3	7.67	5.42	5.86	6.81
VBN	1.60	1.00	1.32	1.76

MCF7, human breast cancer cells; A549, Human lung carcinoma cells; PC3, human prostate carcinoma cells; HepG2, human hepatocellular carcinoma cells. SI is the ratio of the IC<sub>50</sub> values of the compound on PNT2 cells to those in the cancer cell lines.

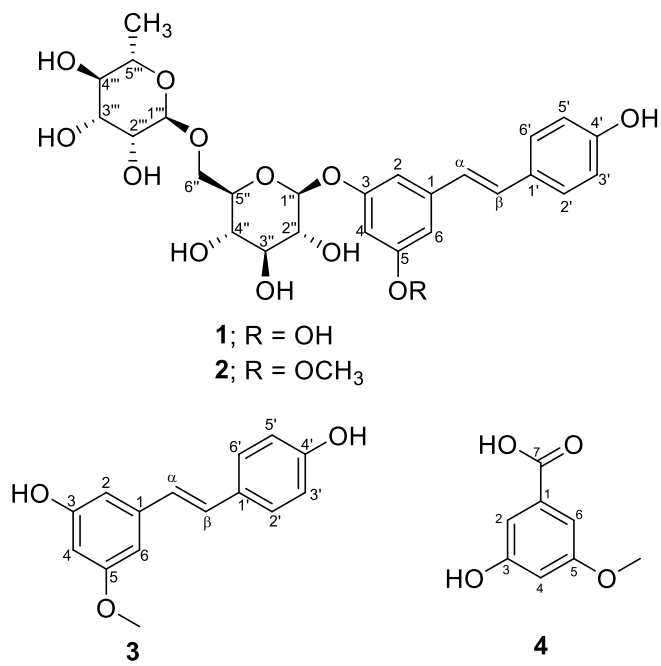


Figure 1. Structure of compounds isolated from the stem bark of *Commiphora africana*

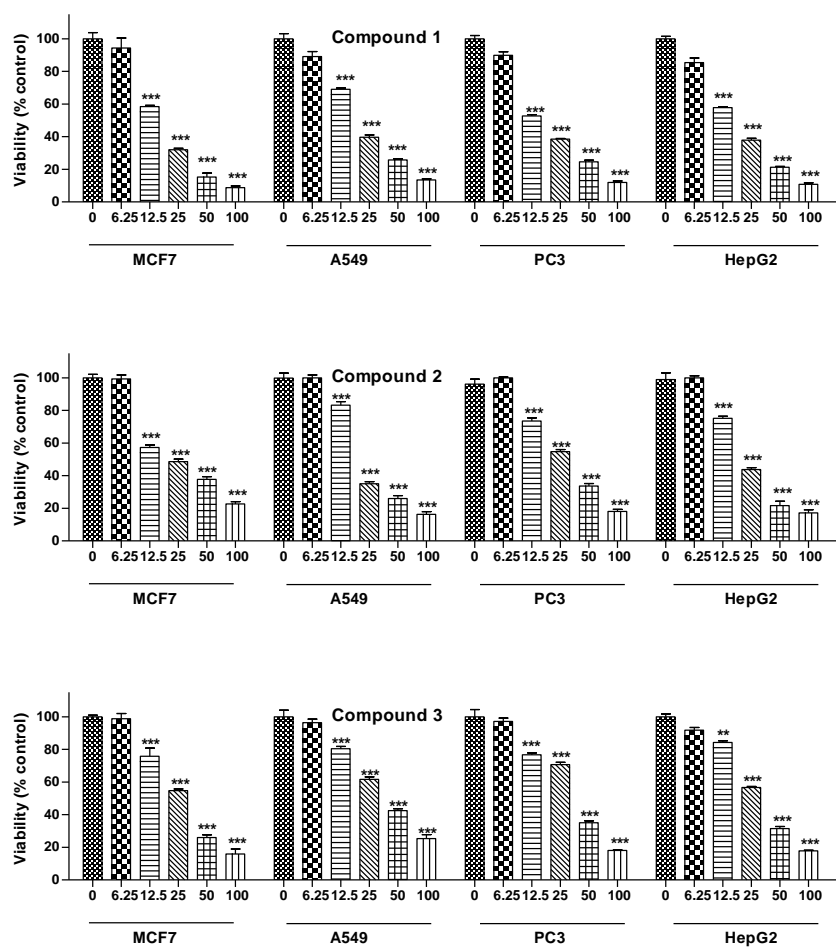


Figure 2. Graphs showing the effect of the test compounds on the viability of MCF7, A549, PC3 and HepG2. The cells were treated with various concentration of compounds for 72 h and the viability was measured using MTT assay. Vinblastine was used as the positive standard, with  $IC_{50}$  of 21 – 37 nM across the panel of carcinoma cells. According to the  $IC_{50}$  value, the rank order of increasing potencies of the compounds is (*E*)-resveratrol 3-*O*-rutinoside (1) > 5-methoxy-(*E*)-resveratrol 3-*O*-rutinoside > pinostilbene (3). Values shown are means  $\pm$  SEM (standard error of the mean) of three different experiments. \*\* $P < 0.01$  and \*\*\* $P < 0.001$ , compared to the negative control (DMSO only), evaluated using the Tukey multiple comparison. X-axis: concentration in  $\mu$ M.

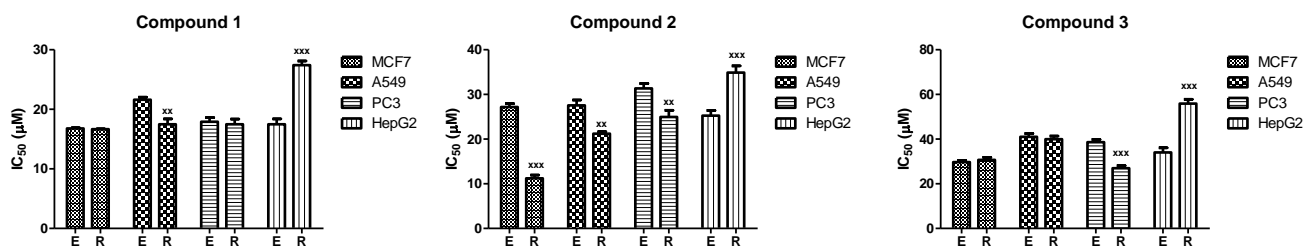


Figure 3: Graphs comparing the IC<sub>50</sub> value of the exposure (E) and recovery assay (R) following the treatment of MCF7, A549, PC3 and HepG2 with various concentrations of the test compounds. Exposure assay measures cell growth inhibition after 72 h treatment, while the recovery assay measures the cell growth inhibition, following replacement of the old medium containing the test compound with fresh growth medium alone for a further 72 h. Vinblastine was used as the positive standard and reference compound. With the exception of the HepG2 cells, the test compounds displayed cytotoxic effects on the cancer cell lines as the IC<sub>50</sub> value in the exposure assay were  $\geq$  those recorded in the recovery assay. Values shown are means  $\pm$  SEM (standard error of the mean) of three different experiments. \*\*P < 0.01 and \*\*\*P < 0.001, compared to the exposure assay, evaluated using the student t-test. X-axis: concentration in  $\mu$ M.