LJMU Research Online

Segun, P, Ogbole, O, Ismail, FMD, Nahar, L, Evans, AR, Ajaiyeoba, E and Sarker, SD

 Bioassay-Guided Isolation and Structure Elucidation of Cytotoxic Stilbenes and Flavonols from the Leaves of Macaranga barteri

http://researchonline.ljmu.ac.uk/id/eprint/10223/

Article

Bioassay-Guided Isolation and Structure Elucidation of Cytotoxic Stilbenes and Flavonols from the Leaves of *Macaranga barteri*

Peter A. Segun[a,b,c], Omonike O. Ogbole[a]*, Fyaz M. D. Ismail[b]*, Lutfun Nahar[b], Andrew R. Evans[b], Edith O. Ajaiyeoba[a] and Satyajit D. Sarker[b]

[a]Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria

[b]Medicinal Chemistry and Natural Products Research Group, School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, James Parsons Building, Byrom Street, Liverpool L3 3AF, United Kingdom

[c]Department of Pharmacognosy, Faculty of Pharmacy, Olabisi Onabanjo University, Sagamu Campus, Nigeria

Corresponding authors

*Tel: +234 805 643 4577, E-mail: nikeoa@yahoo.com (O.O.O)

* Tel: +44 7734 808544, E-mail: fyaz.ismail@gmail.com (F.M.D.I)

ABSTRACT

Bioassay-guided fractionation of the leaves of *Macaranga barteri* collected from Nigeria led to the isolation of three previously undescribed cytotoxic stilbenes, macabartebenes A-C (**1-3**), together with six known compounds including prenylated stilbenes: vedelianin (**4**), schweinfurthin G (**5**), and mappain (**7**), prenylated flavonols: 8-prenylkaempferol (**6**), and broussoflavonol F (**8**), and the geranylated flavonol, isomacarangin (**9**). The cytotoxicity of the compounds was evaluated against four human cancer cell lines, with vinblastine as the positive control and DMSO vehicle as the negative control. Vedelianin ($IC_{50} = 0.32 - 0.54$ µM) displayed the greatest antiproliferative activity across the panel of cancer cell lines amongst the compounds, while macabartebene A ($IC_{50} = 0.60 - 0.79$ µM) was the most potent of the previously unreported compounds. The compounds displayed varying selectivity towards the cancer cell lines compared to the normal human prostate cell line. The findings of this study revealed that *M. barteri* leaves contain several cytotoxic compounds.

Keywords:

Macaranga barteri; Euphorbiaceae; Stilbenes; Flavonols; Macabartebenes A-C; Cytotoxicity; MTT assay

Abbreviations

A549, human lung adenocarcinoma; ANOVA, one-way analysis of variance; ATCC, American type culture collection; CC, column chromatography; CD_3OD, deuterated methanol; COSY, correlated spectroscopy; DCM, dichloromethane; DEPTQ, distortionless enhancement by polarisation transfer; DMSO, dimethyl sulfoxide; ECACC, European collection of authenticated cell cultures; FBS, foetal bovine serum; FHI, forest herbarium Ibadan; HeLa, human cervix adenocarcinoma; HMBC, heteronuclear multiple bond correlation; HPLC, high performance liquid chromatography; HR-EI-MS, high resolution electrospray ionization mass spectrometry; HSQC, homonuclear single quantum correlation; Hz, hertz; IC_{50}; 50% inhibitory concentration; IR, infrared spectroscopy; MCF7, human breast adenocarcinoma; MeOH, methanol; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium; *m/z, m*ass to charge ratio; *n*-hex, *n*-hexane; NOESY, nuclear Overhauser effect spectroscopy; NCI, national cancer institute; NMR, nuclear magnetic resonance; OD, optical density; PBS, phosphate buffered-saline; PC3, human prostate adenocarcinoma; PNT2, normal human immortalised prostate cell line; SEM, standard error of mean; SI, selectivity index; SS, stock solution; TFA, trifluoroacetic acid; UV, ultraviolet spectroscopy; VLC, vacuum liquid chromatography; WHO, world health organisation

1.   Introduction

The genus *Macaranga,* comprising over 300 species, is one of the largest genera of the family Euphorbiaceae and the only genus in the subtribe Macaranginae. These plants are native to South-East Asia, West Africa and the South Pacific region [1]. Plants in this genus are used in folklore medicine for the treatment of boils, cancer, cough, cuts, diarrhoea, hypertension, stomach-ache, swellings and wounds [2-6]. Only about 10% of the plants in this genus have been investigated for their phytochemical constituents and biological activities. Previous phytochemical studies revealed that this genus produces prenylated and geranylated flavonoids, stilbenes, tannins, terpenes, chalcones, coumarins and steroids [7,8]. Several publications from the Laboratory of Drug Discovery Research and Development of the National Cancer Institute have reported the presence of several potent cytotoxic molecules in some members of this genus, especially *M. schweinfurthii* [9-12].

In our search for novel cytotoxic compounds from Nigerian plants, we discovered that the extract of the leaves of *M. barteri* Mull. Arg. displayed potent antiproliferative activity against rhabdomyosarcoma cell line [13,14]. *Macaranga barteri* (synonym; *M. heudelotii* A. Chev.), called '*Agbasa*' or '*Aarasa*' by the Yoruba people of southwestern Nigeria is one of the few members of the *Macaranga* genus available in southwestern Nigeria. Traditional healers in Cameroon, Equatorial Guinea, Ghana, Ivory Coast, Liberia, Nigeria and Sierra Leone utilise the barks and leaves of the plant for the treatment of several ailments including anaemia, bronchitis, cough, fever, gonorrhoea and vermifuge [15,16]. A recent research revealed that the essential oils of the leaves of *M. barteri* contain many constituents that include eremophilene, 6-*epi*-shyobunol and methyl salicylate. Other minor constituents of the oils of this plant include *β*-eudesmene, *allo*-aromadendrene and dihydro-*β*-agarofuran [17]. Previous phytochemical studies focused on the stem bark of *M. barteri* afforded several bioactive compounds including macabarterin, ellagic acid, gallic acid and scopoletin [18].

The present paper describes the bioassay-guided isolation, structure elucidation and antiproliferative activity of three new stilbenes, macabartebenes A-C and six other known compounds (Fig. 1).

2. Materials and methods

2.1.   *General experimental procedures*

UV spectra were recorded on an Analytik Jena UV Visible spectrophotometer (Germany) with MeOH as a solvent. IR spectra were recorded on a PerkinElmer FTIR Spectrum BX spectrometer. 1D and 2D NMR spectra were recorded in CD_3OD using residual solvents as the internal standard on a Bruker Avance III Spectrometer at 600 MHz for ^1H NMR and 150 MHz for ^{13}C NMR, respectively. Quaternary, methine, methylene and methyl carbons were differentiated by DEPT experiments. COSY experiment was used to determine ^1H homonuclear connectivities. HSQC experiment was used to determine ^1H-^{13}C one-bond connectivities experiment while HMBC experiment was used to determine two and three bond connectivities. The chemical shift values (δ) are reported in parts per million (ppm), and the coupling constants value (J) are measured in Hertz (Hz). High resolution mass spectrometric data (HRESIMS) were measured using the Thermo Scientific LTQ Orbitrap XL mass spectrometer at the EPSRC UK National Mass Spectrometry Facility (Swansea, Wales). Solid phase extraction (SPE) was performed on a Strata C-18 cartridge (35μm; 70Å; 20 g). HPLC purification was performed on an Agilent 1260 infinity HPLC System coupled with an Agilent 1260 diode array detector using an Ace-5 C18 column (150×21.2 mm, 5 μm particle size, Hichrom Ltd). HPLC grade solvents were purchased from Fischer Scientific (Loughborough, UK). Absorbance measurement for the MTT assay were taken on a CLARIOstar microplate reader (BMG labtech, Germany). Cell culture materials including Dulbecco's Modified Eagle Medium (DMEM), foetal bovine serum (FBS) and trypsin were obtained from Biosera

(Nauaille, 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).

2.2. Plant material

The leaves of *Macaranga barteri* Mull. Arg. (Euphorbiaceae) were collected in Ibadan, southwestern Nigeria, in June 2016. The plant material was identified and authenticated at Forest Herbarium Unit (FHI), Forestry Research Institute of Nigeria, Ibadan, Nigeria. Voucher specimens (FHI 110626) have been deposited at the FHI.

2.3. Extraction and Isolation

Air-dried and powdered leaves of *M. barteri* (190 g each) were Soxhlet-extracted, sequentially, with *n*-hexane, dichloromethane (DCM) and methanol (MeOH), 800 mL each. To ensure exhaustive extraction, ten cycles were allowed for each extraction, and the heating mantle was kept constant at 50 °C for all extraction. Filtered extracts were dried using a rotary evaporator at 40 °C and stored in the refrigerator prior to use. To facilitate the isolation of the cytotoxic constituents of the DCM extract, it was needful to remove the rich chlorophyll pigment present in the DCM extract. This was achieved by a solid phase extraction (SPE) of this fraction. A Strata C-18 cartridge (35 µm; 70 Å; 20 g) was washed with MeOH (50 mL) followed by equilibration with HPLC grade water (100 mL). The dried DCM fraction (2 g x 2) was dissolved in MeOH (10 mL x 2) and allowed to run through the activated cartridge. The eluate, which was mainly free of the chlorophyll pigment, was dried to give the purified DCM extract (2.91 g). A portion of the purified DCM extract (2 g) was fractionated using SPE. Briefly, 2 g of the purified DCM fraction was dissolved in 10 mL of 20% MeOH-H_2O solution and loaded on to the activated cartridge [19]. The cartridge was eluted with a binary gradient

6

solvent system to obtain seven fractions: 20, 40, 60, 70, 80, 90 and 100% MeOH in water (200 mL each), coded respectively as MBF1, MBF2, MBF3, MBF4, MBF5, MBF6 and MBF7. All seven fractions were evaporated to dryness and stored in the refrigerator prior to cytotoxic screening. Of these, MBF4 and MBF5, which were the most active against the cancer cell lines, were further purified as described below. Each time, UV detection was at 220, 254, and 320 nm, the flow rate was maintained at 10 mL/min. In addition, the injection volume and concentration were maintained at 300 µL and ca. 5 mg/mL, respectively. MBF5 (231 mg) was purified on reverse-phase preparative HPLC (mobile phase: H_2O/MeOH containing 0.1 trifluoroacetic acid (TFA); 0-5 min: 30-85% MeOH, 5-25 min: 80-95% MeOH, 25-30 min: 95-100% MeOH, 30-35 min: 100% MeOH, 35-40 min: 30% MeOH) to afford **1** (13.2 mg; t_R = 8.2 min), **2** (5.7 mg; t_R = 8.9 min), **3** (5.5 mg; t_R = 11.7 min), **4** (5.1 mg; t_R = 15.1 min) and **5** (7.7 mg; t_R = 18.1 min). MBF4 (191 mg) was purified on reversed phase preparative HPLC (mobile phase: H_2O/ACN containing 0.1 trifluoroacetic acid (TFA); 0-4 min: 30-75% ACN, 5-25 min: 75-95% ACN, 25-30 min: 95-100% ACN, 30-35 min: 100% ACN, 35-40 min: 30% ACN) to afford **6** (5.7 mg; t_R = 12.3 min), **7** (6.3 mg; t_R = 13.1 min), **8** (4.9 mg; t_R = 14.7 min) and **9** (16.1 mg; t_R = 17.2 min).

2.3.1. Macabartebene A (*1*)

Amorphous, yellow powder; UV (MeOH) λ_{max} (log ε) 332 (1.50) nm; IR (KBr) ν_{max}3341, 2648, 2161, 1587, 1513, 1432, 1064, 1041 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; positive HRESIMS *m/z* 467.2788 [M+H]$^+$ (calcd for $C_{29}H_{39}O_5$, 467.2792).

2.3.2. Macabartebene B (*2*)

Amorphous, pale yellow powder; UV (MeOH) λ_{max} (log ε) 330 (0.91) nm; IR (KBr) ν_{max}3347, 2323, 1587, 1513, 1436, 1064, 1041 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; positive HRESIMS m/z 467.2789 [M+H]$^+$ (calcd for $C_{29}H_{39}O_5$, 467.2792).

2.3.3   *Macabartebene C (3)*

Amorphous, brown powder; UV (MeOH) λ_{max} (log ε) 202 (2.94), 332 (1.65) nm; IR (KBr) ν_{max}3325, 2161, 1587, 1513, 1437, 1064, 1041 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; positive HRESIMS m/z 467.2788 [M+H]$^+$ (calcd for $C_{29}H_{39}O_5$, 467.2792).

2.4.   *Cell lines and cell culture*

Human adenocarcinoma cell lines: breast (MCF7), cervix (HeLa), lung (A549) and prostate (PC3) were purchased from 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). The cells were cultured in T75 flasks containing DMEM medium supplemented with 10% FBS and 1% penicillin-streptomycin antibiotics suspension. The cells were maintained in a humidified atmosphere containing 5% CO_2 at 37 °C and passaged bi-weekly at a 1:3 dilution using 0.05% trypsin - 0.02% EDTA in PBS. Visual investigation of cells was made using an inverted microscope (Olympus CKX41, UK) at magnifications of 40x and 100x. Cells were counted using an automated haemocytometer (C-Chip NanoEnTek, USA).

2.5.   *Cytotoxicity assay*

After repeated initial experiments, the cell densities for optimal growth were found to be 2000, 4000, 1200, 2000 and 4000 cells/well for MCF-7, HeLa, A549, PC3 and PNT2 respectively. The cell lines were seeded into all wells in a 24 well plate (Corning® Costar®

TC-treated, Merck, UK) and incubated at 37°C/5% CO_2 overnight to allow the cells to adhere to the surface of the plate. After 24 h, the old medium was removed by aspiration, and serial dilutions of extracts/compounds (1 mL each) in DMSO, positive control vinblastine in DMSO or vehicle control (DMSO) were added to quadruplicate wells, and the microtiter plates were incubated for a further 72 h. All dilutions of stock solutions of the extracts/compounds, vinblastine positive control and the DMSO vehicle control were made in cell culture media with the final concentration of DMSO of 0.1%, which had previously been found not to be cytotoxic [19]. At the expiration of the treatment period, the MTT assay was performed to measure the cell viability. The protocol described by Mossman [20] in 1983 was adopted with slight modifications. Briefly, the old medium was removed from each well by aspiration and replaced with 1 mL of 500 µg/mL MTT dye in medium. The plates were incubated for 2 h at 37°C/5% CO_2 to allow the production of formazan crystals. After incubation, the medium was removed by aspiration and 500 µL of propanol to each well was added 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 dilution in each 24-well plate was transferred into a 96-microtitre plate and the absorbance of the solution was measured at 570 nm on a Clario Star microplate reader. The average absorbance reading obtained from the negative control wells was set arbitrarily at 100% and the average absorbance readings of treatment wells was calculated as percentage relative to the negative control. In addition, the same process was carried out for vinblastine (positive control) using the concentration range mentioned above. Each experiment was carried out in quadruplicate on three different occasion and the cell viability was calculated as (A-B) / A, where A and B is the absorbance value of the control and the treated cells, respectively [21]. The IC_{50} (concentration required for the reduction of cell viability by 50%) was estimated using the Microsoft Excel version 2016 from dose−response curves obtained from three independent experiments. The results obtained were expressed as

mean ± standard errors of means (SEM). 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 $P < 0.05$. To obtain the selectivity index (SI) of the isolated compounds, the PNT2 cell line was used. The SI value was calculated as the ratio of the IC_{50} values of the compounds on PNT2 cells relative to those in the cancer cell lines [22].

3.   Results and discussion

Macabartebene A (**1**) was obtained as a yellow, amorphous solid from the CH_2Cl_2 extract of *M. barteri*. The HRESIMS of **1** (Fig. S12, Supporting Information) showed the protonated molecule $[M+H]^+$ at *m/z* 467.2788, corresponding to $C_{29}H_{39}O_5$ (calcd 467.2792) and indicating 11 double-bond equivalents, which is consistent with a stilbene skeleton, with two additional degrees of unsaturation. The ^1H NMR spectrum of **1** (Fig. S2, Supporting Information) of **1** in CD_3OD revealed signals for two *trans* olefinic protons (δ_H 6.82, d (16.0), H-1'; δ_H 6.67, d (16.0), H-2'). In addition, the UV spectrum of **1** (Fig. S13, Supporting Information) showed maximum absorption at 332 nm confirming that the basic skeleton was identical to that of stilbene such as vedelianin [6] and resveratrol [23]. The ^{13}C NMR data of **1** (Table 1) revealed 29 signals comprising eleven quaternary, eight methine, five methylene and five methyl carbon atoms based on the analysis of the DEPTQ and HSQC spectra (Fig. S5-S7, Supporting Information). Further analysis of the ^1H NMR spectrum of **1** (Fig. S2, Supporting Information) showed a symmetrically substituted AA' benzene ring system (ring A) (δ_H 6.45, 2H, s, H-4'and H-8'). Two of the five methyl singlets present in the ^1H NMR spectrum of **1** showed HMBC correlations characteristic of a prenyl side chain. The HMBC correlations from H-4'' (δ_H 1.68, s) to the sp^2 carbons at C-2'' (δ_C 123.2) and C-3'' (δ_C 129.7), the C-5'' (δ_C 16.5) methyl group as

well as COSY correlation between H-1" (δ_H 3.30, d (7.9)) and H-2" (δ_H 5.26, dd (6.4, 1.4)) confirmed the presence of a prenyl moiety. In the HMBC spectrum of **1** (Fig. S9, Supporting Information), the methylene signal at δ_H 3.30 (H-1") showed correlation with C-5', C-7' (δ_C 155.8) and C-6' (δ_C 114.4), indicating that the prenyl chain was linked to the ring A at position C-6'. The ^{1}H NMR spectrum of **1** (Fig. S2, Supporting Information) revealed that the second benzene ring (ring B) of the stilbene was an AB benzene ring system (δ_H 6.84, d (2.2), H-3; δ_H 6.71, d (2.2), H-5). The signal at δ_H 5.38 (H-8, dd (7.8, 1.5)) showed correlation in the HMBC spectrum with the methylene carbon C-7 (δ_C 27.7), the methyl carbon C-10 (δ_C 14.7) and the methylene carbon C-11 (δ_C 39.9), indicating that these signals were associated with the inner prenyl unit. The signal at δ_H 3.34 (H-7, partially obscured by the solvent) showed HMBC correlations with C-1 (δ_C 142.8), C-5 (δ_C 119.2), C-6 (δ_C 128.2), C-8 (δ_C 122.8) and C-9 (δ_C 135.3) indicating that the inner geranyl group was attached to ring B at position C-6. Assignment of the *E* configuration at the C-8:C-9 double bond was deduced from the nuclear Overhauser effect (NOE) correlation between H-10 and H-7 in the 2D NOESY spectrum (Fig. S11, Supporting Information). Quaternary and phenolic carbons were unambiguously assigned by long-range HMBC correlations (H-2' to C-4, H-3 to C-1 and C-2, H-7 to C-6, H-4'/8' to C-6', H-1' to C-3', H-1" to C-5'/7') (Fig. 1). The ^{13}C NMR signal at 70.1 ppm (C-14) indicated that **1** bears an aliphatic hydroxy group. The HMBC correlations of the signal at δ_H 1.46 (H-13, m) with C-11, C-12, and C-14 and the COSY correlations between H-11 (δ_H 2.08, t) and H-12 (δ_H 1.54, m); and H-12 and H-13 (δ_H 1.46, m) indicated the linkage of the hydroxy chain with the inner prenyl unit. The final methyl signals at δ_H 1.19 (6H) showed HMBC correlations to the tertiary alcohol at C-14, as well as to the methylene carbon C-13 (δ_C 42.9), further confirming the structure of this portion of the molecule. In addition, the symmetrical methyl carbons C-15 and C-16 were linked to the tertiary alcohol at C-14**.** On the basis of the foregoing evidences,

the structure of macabartebene A (**1**) was elucidated as 5-((*E*)-3,5-dihydroxy-4-(3-methylbut-2-en-1-yl)styryl)-3-((*E*)-7-hydroxy-3,7-dimethyloct-2-en-1-yl)benzene-1,2-diol.

Macabartebene B (**2**) was obtained as an amorphous pale yellow solid. From the HRESIMS analysis (Fig. S25, Supporting Information), it was determined to have the same protonated molecule ([M+H]$^+$ at *m/z* 467.2789) and molecular formula ($C_{29}H_{39}O_5$) as **1.** The UV spectrum of **2** (Fig. S26, Supporting Information) revealed maximum wavelength of absorption at 330 nm suggesting that the basic stilbene skeleton present in **1** was also available in **2.** The ^{1}H NMR data of **2** (Table 1) was similar to **1**, showing two *trans*-olefinic protons linked to aromatic rings (δ_H 6.83, d (16.2), H-1'; δ_H 6.69, d (16.2), H-2')**,** a symmetrically substituted AA' benzene ring system (ring A) (δ_H 6.47, 2H, s, H-4', H-8') and an AB benzene ring system (δ_H 6.86, d (1.9), H-3; δ_H 6.72, d (1.9), H-5). However, the prenyl unit that was attached to ring A of **1** was absent in **2**. Instead, it was observed from the ^{1}H- and ^{13}C- NMR data (Table 1) that the double bond of the prenyl unit in **1** was hydrated in **2**. This was confirmed by the presence of an aliphatic hydroxy group (C-3'', δ_C 70.5) in **2** that showed HMBC correlation with H-1'' (δ_H 2.69, m), H-2'' (δ_H 1.71, t (7.1)), H-4'' (δ_H 1.28, s) and H-5'' (δ_H 1.28, s) (Fig. 1). The remaining signals in the ^{1}H NMR spectrum of **2** (Fig. S15-S17, Supporting Information) included two olefinic triplets [δ_H 5.38, d (7.2, 1.2), H-8; δ_H 5.16, dd (6.7, 1.4), H-13], three methylene multiplets (δ_H 3.34, H-7; δ_H 2.09, H-11; δ_H 2.16, H-12) and three methyl singlets (δ_H 1.77, H-10; δ_H 1.63, H-15; δ_H 1.68, H-16). Overall, these signals matched very closely those published for the geranyl group of known stilbenes isolated from *M. schweinfurthii.* [9,12]. Furthermore, HMBC resonances from H-8 to C-7 (δ_C 27.6), C-10 (δ_C 14.8) and C-11 (δ_C 39.5) and from H-13 to C-11, C-15 (δ_C 16.5), C-16 (δ_C 24.5) provided further evidence for the presence of the geranyl moiety (Fig. 1). Assignment of the *E* configuration at the C-8:C-9 double bond was deduced from the nuclear Overhauser effect (NOE) correlation between H-10 and H-7 in the 2D NOESY spectrum (Fig. S24, Supporting Information). Quaternary and phenolic carbons were

definitively assigned by long-range HMBC correlations as seen in **1** above. In the HMBC spectrum of **2** (Fig. S21-S23, Supporting Information), the methylene signal at δ_H 3.34 (H-7) showed correlation with C-1 (δ_C 142.8), C-5 (δ_C 119.2), C-6 (δ_C 128.2), C-8 (δ_C 122.7) and C-9 (δ_C 135.2), indicating that the geranyl chain was attached to ring B at position C-6. On the basis of these data, the structure of macabartebene B (**2**) was elucidated as 5-((*E*)-3,5-dihydroxy-4-(3-hydroxy-3-methylbutyl)styryl)-3-((*E*)-3,7-dimethylocta-2,6-dien-1-yl)benzene-1,2-diol.

Macabartebene C (**3**) was obtained as an amorphous brown solid. A molecular formula of $C_{29}H_{38}O_5$ was deduced from the HRESIMS of **3** (Fig. S38, Supporting Information), which presented the protonated molecule at *m/z* 467.2788 [M+H]$^+$ (calcd 467.2792). The molecular formula of **3** was similar to that of **1** and **2** and also suggested 11 degrees of unsaturation. The ^{13}C NMR data of **3** (Table 1) revealed 29 signals comprising eleven quaternary, eight methine, five methylene and five methyl carbon atoms based on the analysis of the DEPTQ and HSQC spectra (Fig. S32-S34, Supporting Information). The ^1H NMR spectrum of **3** (Fig. S28-30, Supporting Information) revealed the presence of two *trans*-olefinic protons attached to an aromatic ring (δ_H 6.84, d (16.3), H-1'; δ_H 6.71, d (16.3), H-2'), which confirms the stilbene basic skeleton of **3.** This was corroborated by the UV spectrum of **3** (Fig. S39, Supporting Information) that showed maximum absorption at 332 nm, which is typical of stilbenes. Further examination of the ^1H- and ^{13}C- HMR spectra (Fig. S28-S31, Supporting Information) revealed that the stilbene was made up of two AB benzene ring systems; ring A (δ_H 6.46, d (1.4), H-4'; δ_H 6.39, d (1.4), H-8') and ring B (δ_H 6.86, d (1.9), H-3; δ_H 6.73, d (1.9), H-5). Key HMBC and COSY correlations and the presence of an aliphatic hydroxy group (C-3", δ_C 74.8) in **3** revealed that the hydrated prenyl moiety present in **2** was also present in **3** (Fig. 1). However, unlike **2** where the moiety attached to ring A at position C-6', the hydrated prenyl group was linked to ring A of **3** at position C-7'. This position further supported the lack of symmetry in ring A of

3 that was observed in ring A of **2**. Careful examination of the remaining signals on the ^{1}H- and ^{13}C- NMR spectra (Fig. S28-S32, Supporting Information) together with HMBC and COSY spectra (Fig. S33-S37, Supporting Information) confirmed the presence of a geranyl moiety, which was linked to ring B at position C-6, like what was observed in **2**. The location of the hydrated prenyl group turned out to be the only difference between **2** and **3**. According to these spectral data, the structure of macabartebene C (**3**) was elucidated as 5-((*E*)-3,4-dihydroxy-5-(3-hydroxy-3-methylbutyl)styryl)-3-((*E*)-3,7-dimethylocta-2,6-dien-1-yl)benzene-1,2-diol.

The known compounds vedelianin (**4**) [6], schweinfurthin G (**5**) [24], 8-prenylkaempferol (**6**) [25], mappain (**7**) [26], broussoflavonol F (**8**) [27], and isomacarangin (**9**) [11] were determined by the comparison of their spectroscopic data with those reported in the literature (Fig. S43; Supporting Information). All the compounds were isolated from *M. barteri* for the first time.

Table 1. ^{1}H (600 MHz, CD$_3$OD) and ^{13}C (150 MHz, CD$_3$OD) NMR data of compounds **1−3**

Position	1		2		3	
	^{1}H (J in Hz)	^{13}C, mult.	^{1}H (J in Hz)	^{13}C, mult.	^{1}H (J in Hz)	^{13}C, mult.
1		142.8, C		142.8, C		144.3, C
2		144.6, C		144.7, C		146.1, C
3	6.84, d (2.2)	109.6, CH	6.86, d (1.9)	109.6, CH	6.86, d (1.9)	111.0, CH
4		129.0, C		129.0, C		129.6, C
5	6.71, d (2.2)	119.2, CH	6.72, d (1.9)	119.2, CH	6.73, d (1.9)	120.7, CH
6		128.2, C		128.2, C		129.2, C
7	3.34, obs^b	27.7, CH$_2$	3.34, obs^b	27.6, CH$_2$	3.34, obs^b	29.0, CH$_2$
8	5.38, dd (7.8, 1.5)	122.8, CH	5.38, dd (7.2, 1.2)	122.7, CH	5.38, dd (7.6, 1.2)	124.2, CH
9		135.3, C		135.2, C		136.6, C
10	1.76, s	14.7, CH$_3$	1.77, s	14.8, CH$_3$	1.76, s	16.2, CH$_3$
11	2.08, dd (7.4, 1.4)	39.9, CH$_2$	2.09, m	39.5, CH$_2$	2.09, m	40.5, CH$_2$
12	1.54, m	22.3, CH$_2$	2.16, m	26.4, CH$_2$	2.16, m	27.8, CH$_2$
13	1.46, m	42.9, CH$_2$	5.16, dd (6.7, 1,4)	124.0, CH	5.16, dd (7.0, 1,4)	125.4, CH
14		70.1, C		130.9, C		132.2, C
15	1.19, s	27.8, CH$_3$	1.63, s	16.5, CH$_3$	1.63, s	17.8, CH$_3$
16	1.19, s	27.8, CH$_3$	1.68, s	24.5, CH$_3$	1.68, s	25.9, CH$_3$
1'	6.82, d (16.0)	127.6, CH	6.83, d (16.2)	127.6, CH	6.84, d (16.3)	129.3, CH
2'	6.67, d (16.0)	125.6, CH	6.69, d (16.2)	125.5, CH	6.71, d (16.3)	126.8, CH
3'		136.3, C		136.3, C		138.3, C
4'	6.45, s	104.3, CH	6.47, s	103.1, CH	6.46, d (1.4)	104.5, CH
5'		155.8, C		155.9, C		156.2, C
6'		114.4, C		115.3, C		156.8, C
7'		155.8, C		155.9, C		109.3, C
8'	6.45, s	104.3, CH	6.47, s	103.1, CH	6.39, d (1.4)	107.8, CH
1"	3.30, d (7.9)	21.9, CH$_2$	2.69, m	18.0, CH$_2$	2.66, m	17.8, CH$_2$
2"	5.26, dd (6.4, 1.4)	123.2, CH	1.71, t (7.1)	42.1, CH$_2$	1.81, t (7.1)	33.5, CH$_2$
3"		129.7, C		70.5, C		74.8, C
4"	1.68, s	24.6, CH$_3$	1.28, s	25.5, CH$_3$	1.33, s	26.9, CH$_3$
5"	1.78, s	16.5, CH$_3$	1.28, s	27.7, CH$_3$	1.33, s	26.9, CH

The cytotoxicity of the extracts, fractions and all isolated compounds was determined, using the MTT assay, against MCF7 (human breast adenocarcinoma), HeLa (human cervix adenocarcinoma), A549 (human lung adenocarcinoma) and PC3 (human prostate carcinoma) cell lines. These cell lines were chosen for this study because of the high prevalence of these cancers and the need to develop effective and safe chemotherapeutic agents. As an illustration, the breast and cervical cancers are the commonest form of cancer in women, while the prostate cancer is the most frequently diagnosed cancer type in men. In addition, lung cancer was the commonest cause of cancer mortality globally in 2015, accounting for 18.2% (1.7 million deaths) of the total cancer mortality [28]. Among the extracts, the CH_2Cl_2 extract displayed the highest cytotoxicity with its greatest effect on the breast carcinoma ($IC_{50} = 2.5$ µg/mL) and the lowest effect on the prostate carcinoma cells ($IC_{50} = 3.9$ µg/mL) (Table S1; Supporting Information). This fraction accounted for the antiproliferative activities of this extract and was therefore subjected to bioassay-guided fractionation. Among the seven sub-fractions (MBF1 – MBF7) obtained from solid phase extraction (SPE) fractionation, only MBF4 and MBF5 retained the antiproliferative activity of the extract as indicated by their low IC_{50} value (Table S1; Supporting Information). Chromatographic separation of MBF4 and MBF5 using reversed-phase preparative HPLC led to the isolation of compounds **1-9.** Since stilbenes and flavonoids have been reported to exhibit moderate or strong antiproliferative activities [29,30], the cytotoxicity of compounds **1-9** were evaluated against four human cancer cell lines, with vinblastine as the positive control and DMSO vehicle as the negative control. The results of the cytotoxicity of compounds **1-9** are shown in Table 2.

Table 2: Antiproliferative Effects of compounds **1-9** Isolated from the Leaves of *Macaranga barteri* against Four Human Cancer Cell Lines[a] and the Normal Human Prostate Cell Line

| Compound | IC_{50} (µM)[b] | | | | |
	MCF7	A549	PC3	HeLa	PNT2
1	0.68 ± 0.01	0.79 ± 0.01	0.66 ± 0.01	0.60 ± 0.01	9.08 ± 0.11
2	0.71 ± 0.02	0.74 ± 0.01	0.69 ± 0.01	0.72 ± 0.01	11.12 ± 0.11
3	1.73 ± 0.01	1.81 ± 0.00	1.61 ± 0.01	1.67 ± 0.01	10.81 ± 0.17
4	0.32 ± 0.03	0.54 ± 0.02	0.39 ± 0.01	0.51 ± 0.01	3.76 ± 0.12
5	0.95 ± 0.02	1.10 ± 0.09	0.91 ± 0.01	1.18 ± 0.01	13.61 ± 0.41
6	6.22 ± 0.13	6.61 ± 0.21	6.53 ± 0.11	6.88 ± 0.16	27.06 ± 0.06
7	0.71 ± 0.02	0.81 ± 0.02	0.77 ± 0.01	0.71 ± 0.01	12.94 ± 0.06
8	4.13 ± 0.00	3.83 ± 0.01	3.99 ± 0.01	4.10 ± 0.01	22.31 ± 0.13
9	8.43 ± 0.26	8.72 ± 0.21	8.5 ± 0.31	8.49 ± 0.21	25.11 ± 0.43
Vinblastine	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.06 ± 0.01

[a]MCF7, human breast adenocarcinoma; A549, human lung carcinoma; PC3, human prostate adenocarcinoma; HeLa = human cervix adenocarcinoma; PNT2 = normal human immortalised prostate cell line. [b]MTT method, with the cells incubated with the test compounds for 72 h (means ± SEM, n = 3).

All the test compounds displayed concentration-dependent activity across the four human carcinoma cell lines (Supplementary Fig. S41 and S43), although with varying degrees of potency as revealed by their IC_{50} values (Table 2). The isolated compounds could be classified into two classes; stilbenes (**1**-**5, 7**) and flavonols (**6, 8**-**9**). It was observed that the stilbenes showed potent cytotoxicity, while the flavonols displayed moderate cytotoxicity against the cancer cell line under investigation. Amongst the stilbenes, vedelianin (**4**) displayed the highest cytotoxicity. Comparing the cytotoxicities of vedelianin with those of schweinfurthin G (**5**) across the panel of carcinoma cells under investigation (Table 3), it was observed that vedelianin was almost thrice as potent as schweinfurthin G, suggesting that the C-3 hydroxy group enhances the activity of vedelianin. A previous studies comparing the antiproliferative

activities of vedelianin with schweinfurthin G on the A2780 human ovarian cancer cell line, a similar result was observed [24]. It is interesting to mention that vedelianin (**4**) and schweinfurthin G (**5**) are structurally related to cyclocannabigerols A and B, cyclized isoprenoid cannabinoids isolated *Cannabis* that demonstrated remarkable modulation of the transient receptor potential (TRP) proteins [31]. Macabartebenes A (**1**) and B (**2**) which have hydrated prenyl side chain, compared to mappain (**7**), have similar IC_{50} values with mappain, across the cell line under investigation. This suggest that hydration of the geranyl side-chain of mappain does not markedly affect its cytotoxicity. This is in line with an earlier observation by Beutler *et al.,* who reported that schweinfurthin D, containing a hydrated geranyl moiety, was found to be equipotent with schweinfurthin B against SF295 (brain carcinoma cells) and A549 (lung carcinoma cells) [10]. However, the symmetrically substituted AA' benzene ring system of ring A seems to be expedient for the cytotoxicity of both macabartebenes A and B, as macabartebene B (**3**) which has a hydrated prenyl side-chain at position C-7' had a lower cytotoxicity, compared to its two other structural isomers.

Vedelianin, mappain and the schweinfurthin class of stilbenes have been widely investigated for their antiproliferative activity on various cancer cell lines, especially by several researchers at the National Cancer Institute (NCI) [10,12,24,9,26]. Also, several studies have been undertaken to evaluate the biological activities of many schweinfurthin analogues in order to develop the structure-activity relationships which will guide in the design of drug leads for their putative molecular target [32,33]. In particular, vedelianin was reported to be the most potent antiproliferative agents amongst group of naturally occurring stilbenes with IC_{50} of 130 nM against A2780 cells, mean GI_{50} of 78 nM in the 60 cell line assay of the NCI and GI_{50} value below 1 nM in several other individual cell lines [24]. This intriguing antiproliferative activity of vedelianin (**4**) has necessitated the recent total synthesis of vedelianin in order to further examine its biological activity *in vivo* [34]. This is the first report of the isolation of stilbenes

from *M. barteri* although vedelianin, mappain and schweinfurthin G have been isolated from other species of the *Macaranga* genus.

In the present study, it was observed that broussoflavonol F (**8**) displayed the highest cytotoxicity (IC$_{50}$ value of 3.83 – 4.13 μM) across the cell lines amongst the flavonols. It was also observed that 8-prenylkaempferol (**6**) that lacks the prenyl group present in position C-3 of broussoflavonol had a lower cytotoxicity (IC$_{50}$ value of 6.22 – 6.88 μM) than broussoflavonol. Isomacarangin (**9**) contained a geranyl group in position C-8, unlike the prenyl moiety present in 8-prenylkaempferol. This led to a slight reduction in antiproliferative activities (IC$_{50}$ value 8.43 – 8.72 μM) against the carcinoma cell lines. To the best of the knowledge in available literature, this is the first report of the isolation of these three flavonoids from *M. barteri* and their cytotoxicity. The *Macaranga* genus has been reported to be rich in cytotoxic flavonoids, especially prenylated and geranylated flavonoids [3,35,36]. Potent cytotoxic flavonoids from this genus include malaysianone A, 6-prenyl-3′-methoxyeriodictyol, nymphaeols B-C from *M. trilobal*; kurzphenols B-C from *M. kurzii*; denticulatins C-E from *M. denticulata*; and macarindicins A–C from *M. indica* [36,37,35,38].

The ultimate goal of cancer chemotherapy is the ability of anticancer agents to differentiate between cancerous and normal human cell line. Chemotherapeutic agents are expected to display selective toxicity as this is needful to overcome noxious side effects associated with usage. To measure the selectivity index (SI) of the isolated compounds used in this study, the normal human prostate cells (PNT2) was used. Vinblastine, the positive control, had a SI value ranging from 1.00 to 1.76 across the cancer cell lines, indicating that the drug cannot sufficiently differentiate between normal and cancer cells. This lack of cancer cell specificity of vinblastine was also reported in previous work [22,39]. It is interesting to note that apart from **9,** the cytotoxicities of these compounds were significantly lower towards the normal human cell line compared to the cancer cells (Table 3). The stilbenes, which displayed potent

cytotoxicities against the cell lines, also showed better SI, with mappain (**7**) exhibiting approximately 15- to 18- fold higher IC_{50} value against the carcinoma cell lines compared to PNT2. On the other hand, the flavonols showed lower SI across the cell lines under investigation. In fact, **9** displayed SI value that is similar to that of vinblastine, indicating that it is only two to three times more cytotoxic to the cancer cells than the PNT2 cells.

Table 3. The Selectivity Index (SI) of Compounds **1-9** and Vinblastine (VBN)

Compound	MCF7	A549	PC3	HeLa
1	13.35	11.49	13.75	15.13
2	15.66	15.02	16.11	15.44
3	6.24	5.97	6.71	6.47
4	11.75	6.96	9.64	7.37
5	14.32	12.37	14.95	11.53
6	4.35	4.09	4.14	3.93
7	18.22	15.97	16.80	18.22
8	5.40	5.82	5.59	5.44
9	2.97	2.87	2.95	2.95
Vinblastine	1.60	1.00	1.32	1.76

MCF7, human breast cancer cells; A549, Human lung carcinoma cells; PC3, human prostate carcinoma cells; HeLa, human cervix carcinoma cells. SI is the ratio of the IC_{50} values of the compound on PNT2 cells to those in the cancer cell lines.

Recent studies have revealed the promising antibacterial, antifungal, antiprotozoal and antiviral activities of the extract of the leaves of *M. barteri,* and stated that at the tested doses, the extract could be considered relatively safe and non-toxic [40-42]. The antiproliferative activity of the DCM fraction of *M. barteri* reported herein and the abundance of cytotoxic compounds in this plant may provide evidence for its use in folklore medicine for the treatment of various cancers.

3. Conclusion

To summarise our findings, the phytochemical investigations of the leaves of *Macaranga barteri* led to the isolation and structure elucidation of six stilbenes (**1-5**, **7**) including three previously unreported ones (**1-3**) and three flavonols (**6**, **8-9**). Their cytotoxicity against human breast adenocarcinoma (MCF7), human cervix adenocarcinoma (HeLa), human lung adenocarcinoma (A549) and human prostate adenocarcinoma (PC3) were evaluated. Overall, the stilbenes possess more cytotoxic activity than the flavonols with vedelianin (**4**) being the most cytotoxic across the panel of cancer cell lines amongst the compounds, while macabartebene A (**1**) was the most potent of the new compounds. The antiproliferative activity of the leaves of *M. barteri* reported in this study may provide evidence for its use in folklore medicine for the treatment of various cancers.

References

[1] F.R. Blattner, K. Weising, G. Bänfer, U. Maschwitz, B. Fiala, Molecular analysis of phylogenetic relationships among myrmecophytic *Macaranga* species (Euphorbiaceae). Mol. Phylogen. Evo. 19 (2001). 331-344.

[2] T. Lim, Y. Lim, C. Yule, Evaluation of antioxidant, antibacterial and anti-tyrosinase activities of four *Macaranga* species. Food Chem. 114 (2009) 594-599.

[3] D.S. Jang, M. Cuendet, M.E. Hawthorne, L.B. Kardono, K. Kawanishi, H.H. Fong, R.G. Mehta, J.M. Pezzuto, A.D. Kinghorn, Prenylated flavonoids of the leaves of *Macaranga conifera* with inhibitory activity against cyclooxygenase-2. Phytochemistry 61 (2002) 867-872.

[4] P. Pailee, S. Sangpetsiripan, C. Mahidol, S. Ruchirawat, V. Prachyawarakorn, Cytotoxic and cancer chemopreventive properties of prenylated stilbenoids from *Macaranga siamensis*. Tetrahedron 71 (2015) 5562-557

[5] A. Nick, T. Rali, O. Biological screening of traditional medicinal plants from Papua New Guinea. J. Ethnopharmacol. 49 (1995) 147-156.

[6] O. Thoison, E. Hnawia, F. Guiéritte-Voegelein, T. Sévenet, Vedelianin, a hexahydroxanthene derivative isolated from *Macaranga vedeliana*. Phytochemistry 31 (1992) 1439-1442

[7] J.J. Magadula, Phytochemistry and pharmacology of the genus Macaranga: A review. J. Med. Plants Res. 8 (2014) 489-503.

[8] C. Lei, L.B. Zhang, J. Yang, L.X. Gao, J.Y. Li, J. Li, A.J. Hou, Macdentichalcone, a unique polycyclic dimeric chalcone from *Macaranga denticulata*. Tetra. Lett. 57 (2016) 5475-5478.

[9] J.A. Beutler, R.H. Shoemaker, T. Johnson, M.R. Boyd, Cytotoxic geranyl stilbenes from *Macaranga schweinfurthii*. J. Nat. Prod. 61 (1998) 1509-1512.

[10] J.A. Beutler, J. Jato, G.M. Cragg, M.R. Boyd, Schweinfurthin D, a cytotoxic stilbene from *Macaranga schweinfurthii*. Nat. Prod. Lett. 14 (2000) 399-404.

[11] J.A. Beutler, K.L. McCall, M.R. Boyd, A novel geranylflavone from *Macaranga schweinfurthii*. Nat. Prod. Lett. 13 (1999) 29-32.

[12] P. Klausmeyer, Q.N. Van, J. Jato, T.G. McCloud, J.A. Beutler, Schweinfurthins I and J from *Macaranga schweinfurthii*. J. Nat. Prod. 73 (2010) 479-481.

[13] O.O. Ogbole, P.A. Segun, A.J. Adeniji, In vitro cytotoxic activity of medicinal plants from Nigeria ethnomedicine on Rhabdomyosarcoma cancer cell line and HPLC analysis of active extracts. BMC Comp. Alt. Med. 17 (2017), 494.

[14] P.A. Segun, O.O. Ogbole, E.O. Ajaiyeoba, Medicinal Plants Used in the Management of Cancer among the Ijebus of Southwestern Nigeria. J. Herb. Med. 14, 2018, 68-75.

[15] S. Adesegun, N. Elechi, H. Coker, Antioxidant power of *Macaranga barteri* leaf. Am. J. Food Tech. 2 (2007) 543-549.

[16] H.M. Burkill, The useful plants of west tropical Africa. Volume 2: Families EI. Royal Botanic Gardens (1994)

[17] A.L. Ogundajo, R.O. Olawunmi, C.O. Nnaemeka, I.A. Ogunwande, Essential oil constituents of *Macaranga barteri*. Chem. Nat. Comp. 53 (2017), 971-973.

[18] R.M. Ngoumfo, G.E. Ngounou, C.V. Tchamadeu, M.I. Qadir, C.D. Mbazoa, A. Begum, F.N. Ngninzeko, D. Lontsi, M.I. Choudhary, nhibitory effect of macabarterin, a polyoxygenated ellagitannin from *Macaranga barteri*, on human neutrophil respiratory burst activity. J. Nat. Prod. 71 (2008) 1906-1910.

[19] N. Basar, O.A. Oridupa, K.J. Ritchie, L. Nahar, N.M.M. Osman, A. Stafford, H. Kushiev, A. Kan, S.D. Sarker, Comparative cytotoxicity of *Glycyrrhiza glabra* roots from different

geographical origins against immortal human keratinocyte (HaCaT), lung adenocarcinoma (A549) and liver carcinoma (HepG2) Cells. Phytother. Res. 29 (2015) 944-948.

[20] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65 (1983) 55-63.

[21] O. Ogbole, P. Segun, T. Akinleye, P. Fasinu, Antiprotozoal, antiviral and cytotoxic properties of the Nigerian Mushroom, *Hypoxylon fuscum* Pers. Fr. (Xylariaceae). Acta Pharm. Sci. 56 (2018) 43-56.

[22] P.A. Segun, F.M. Ismail, O.O. Ogbole, L. Nahar, A.R. Evans, E.O. Ajaiyeoba, S.D. Sarker, Acridone alkaloids from the stem bark of *Citrus aurantium* display selective cytotoxicity against breast, liver, lung and prostate human carcinoma cells. J. Ethnopharmacol. 227 (2018) 131–138.

[23] F. Orsini, F. Pelizzoni, B. Bellini, G. Miglierini, Synthesis of biologically active polyphenolic glycosides (combretastatin and resveratrol series). Carbohydrate Res. 301 (1997) 95-109.

[24] B.J. Yoder, S. Cao, A. Norris, J.S. Miller, F. Ratovoson, J. Razafitsalama, R. Andriantsiferana, V.E. Rasamison, D.G. Kingston, Antiproliferative prenylated stilbenes and flavonoids from *Macaranga alnifolia* from the Madagascar rainforest. J. Nat. Prod. 70 (2007) 342-346.

[25] P.I. Hillerns, M. Wink, Binding of flavonoids from Sophora flavescens to the rat uterine estrogen receptor. Planta Med. 71 (2005), 1065-1068.

[26] J.E. van der Kaaden, T.K. Hemscheidt, S.L. Mooberry, Mappain, a new cytotoxic prenylated stilbene from *Macaranga mappa*. J. Nat. Prod. 64 (2001) 103-105.

[27] S.C. Fang, B.J. Shieh, R.R. Wu, C.N. Lin, Isoprenylated flavonols of formosan *Broussonetia papyrifera*. Phytochemistry 38 (1995) 535-537.

[28] C. Global Burden of Disease Cancer, Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 32 cancer groups, 1990 to 2015: A systematic analysis for the global burden of disease study. JAMA Oncol. 3 (2017) 524-548.

[29] B. Bruno, V. Alberto, M. Pilar, M. Domenico, M. Giuliano Delle, Prenylated Flavonoids: Pharmacology and Biotechnology. Curr. Med. Chem. 12 (2005), 713-739.

[30] T. Shen, X.N. Wang, H.X. Lou, Natural stilbenes: an overview. Nat. Prod. Rep. 26 (2009) 916-935.

[31] A. Lopatriello, D. Caprioglio, A. Minassi, A.S. Moriello, C. Formisano, L. De Petrocellis, G. Appendino, O. Taglialatela-Scafati, Iodine-mediated cyclization of cannabigerol (CBG) expands the cannabinoid biological and chemical space. Bioorg. and med. Chem. 26 (2018), 4532-4536.

[32] J. Neighbors, M. Salnikova, J. Beutler, D. Wiemera, Synthesis and structure–activity studies of schweinfurthin B analogs: Evidence for the importance of a D-ring hydrogen bond donor in expression of differential cytotoxicity. Bioorg. Med. Chem. 14 (2006) 1771-1784.

[33] N. Mente, A. Wiemer, J. Neighbors, J. Beutler, R. Hohld, D. Wiemera, Total synthesis of (R,R,R)- and (S,S,S)-schweinfurthin F: Differences of bioactivity in the enantiomeric series. Bioorg. Med. Chem. Lett. 17 (2007) 911-915.

[34] J. Topczewski, D. Wiemer, First total synthesis of (+)-vedelianin, a potent antiproliferative agent. Tetra. Lett. 52 (2011) 1628-1630.

[35] I. Zakaria, N. Ahmat, F. Jaafar, A. Widyawaruyanti, Flavonoids with antiplasmodial and cytotoxic activities of *Macaranga triloba*. Fitoterapia 83 (2012) 968–972.

[36] Y. Da-Song, W. Jian-Guo, P. Wei-Bing, S. Chen, Y. Yong-Ping, L. Ke-Chun, L. Xiao-Li, Cytotoxic prenylated bibenzyls and flavonoids from *Macaranga kurzii*. Fitoterapia 99 (2014) 261-266.

[37] Y. Da-Song, P. Wei-Bing, Y. Yong-Ping, L. Ke-Chun, L. Xiao-Li, X. Wei-Lie, Cytotoxic prenylated flavonoids from *Macaranga indica.* Fitoterapia 103 (2015) 187-191.

[38] Y. Da-Song, L. Zi-Lei, P. Wei-Bing, Y. Yong-Ping, W. Xue, L. Ke-Chun, L. Xiao-Li, X. Wei-Lie, Three new prenylated flavonoids from *Macaranga denticulata* and their anticancer effects. Fitoterapia 103 (2015) 165-170

[39] P.A. Segun, O.O. Ogbole, F.M. Ismail, L. Nahar, A.R. Evans, E.O. Ajaiyeoba, S.D. Sarker, Resveratrol derivatives from *Commiphora africana* (A. Rich.) Endl. display cytotoxicity and selectivity against several human cancer cell lines. Phytother Res. **33** 2018, (1), 159-166

[40] A. Ogundajo, B. Okeleye, A.O. Ashafa, Chemical constituents, *in vitro* antimicrobial and cytotoxic potentials of the extracts from *Macaranga barteri* Mull-Arg. Asian Pac. J. Trop. Biomed. 7 (2017) 654-659.

[41] O. Ogbole, P. Segun, P. Fasinu, Antimicrobial and antiprotozoal activities of twenty-four Nigerian medicinal plant extracts. S. Afr. J. Bot. 117 (2018) 240-246.

[42] O.O. Ogbole, T.E. Akinleye, P.A. Segun, T.C. Faleye, A.J. Adeniji, In vitro antiviral activity of twenty-seven medicinal plant extracts from Southwest Nigeria against three serotypes of echoviruses. Virol. J. 15 (2018) 110.