Antioxidant and cytotoxicity activities of δ-tocotrienol from the seeds of Allophylus africanus

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ABSTRACT
Chemical investigation of Allophylus africanus P. Beauv fruits led to the isolation of a new δ-tocotrienol, 3α-hydroxy-δ-tocotrienol (1) together with eight known compounds (2-9). Compound (1) was allylated (1a) and prenylated (1b and 1c) to give three new semi-synthesized derivatives which were fully characterized as: 6-O-allyl-3α-hydroxy-δ-tocotrienol (1a), 6-O-prenyl-3α-hydroxy-δ-tocotrienol (1b) and 6-O,5-C-diprenyl-3α-hydroxy-δ-tocotrienol (1c). The structures of compounds were established using comprehensive spectroscopic analysis including UV, MS, 1D NMR, 2D NMR and by comparison with the corresponding literature data. Compound (1) and its semi-synthetic derivatives (1a-c) were tested for their antioxidant activity using DPPH radical scavenging assay and also for their cytotoxicity using human cervix carcinoma KB-3-1 cell lines. The results showed that compound (1) exhibited antioxidant activity with an IC_{50} value of 0.25 μM compared to the reference control trolox (26 μM); and good cytotoxic activity with IC_{50} values of 97 μM compared to the reference (+)-griseofulvin (IC_{50} between 17-21 μM).

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1. Introduction

Allophylus is one of the largest genera belonging to the Sapindaceae family with about 255 species and are widely distributed in subtropical and tropical regions of Africa, America, Asia, India Archipelago, Australia, Pacific and Madagascar (Piaggio and Delfino 2014; Fatima 2016). Allophylus africanus P. Beauv is one of the most important conserved and valued forest species in Africa due to their multiple uses, high nutritional content (Oladosu et al. 2015; Martin and Martin 2009; Onuminya and Ogundipe 2014) and its medicinal values. The plant is used in the treatment of conjunctivitis, dysentery, arthritic conditions, malaria, skin inflammation and eyes trouble (Burkill 1983; Oladosu et al. 2013; Catarino et al. 2016; Biseko et al. 2019). The plant is also used as antipyretic, anti-diarrhoea, anticancer, anti-headache, anti-migraine and has vermicidal properties (Burkill 1985; Gathirwa et al. 2011; Oladosu et al. 2013; 2015; Biseko et al. 2019). Previous phytochemical studies on the whole plant of A. africanus reported the isolation of terpenoids (Oladosu et al. 2015); while in other Allophylus species C-glycosyl flavonoids, sesquiterpenoids, coumarins, vanillic acid derivatives, triterpenoids, as well as ceanothane-type triterpenoids, tocotrienols and fatty acids were reported (Hoffmann-Bohm et al. 1992; David et al. 2004; Ciepichal et al. 2007; Diaz et al. 2008; Kumar et al. 2010; Zhang et al. 2012; Balogun et al. 2016; Sangsopha et al. 2020). Some exhibited strong antimalarial, antibacterial, anticancer and antioxidant activities (Sofidiya et al. 2007; Oladosu et al. 2014; Balogun et al. 2016). Despite the studies already done on the different parts of A. africanus, no chemical and/or biological study have yet been carried out on the fruits. In our continuing search for bioactive compounds from plants, we investigated the fruits of A. africanus and herein report on the isolation and structure elucidation of a new δ-tocotrienol; 3α-hydroxy-δ-tocotrienol together with its DPPH antioxidant and cytotoxic assays.

2. Results and discussion

The dry powdered fruits of A. africanus were extracted with MeOH. Repeated and successive column chromatography on the MeOH extract led to the isolation and
characterization of one new compound (1) (Figure 1) together with eight known secondary metabolites namely; allantoin (2) (Wang and Wang 2018), shikimic acid (3) (Saito et al. 1997), scopoletin (4) (Khan and Hossain 2015), eicosanoic acid (5) (Makhafola et al. 2017), pelargonic acid (6) (Kramer et al. 1998), lauric acid (7) (Kramer et al. 1998), β-sitosterol (8) and β-sitosterol 3-O-β-D-glucopyranoside (9) (Khatun et al. 2012) (Figure S14).

Compound 1 was obtained as a white amorphous solid with $[\alpha]_{D}^{20} = -1.3$ (C = 1.22 mg/mL, CH$_2$Cl$_2$). Its UV-visible spectrum showed an absorption band at 304 nm characteristic of tocols (Lampi 2011). Its molecular formula was determined as C$_{27}$H$_{40}$O$_3$ from HRESIMS in the positive mode where an pseudomolecular ion [M + H]$^+$ at m/z 413.3050 (calcd 413.30502 for C$_{27}$H$_{41}$O$_3$)$^+$ was found. The $^1$H NMR spectrum exhibits a set of signals including those of two aromatic meta-coupled protons resonating at $d_{H}$ 6.52 (1H, $d$, $J$ = 3.0 Hz, H-7) and 6.35 (1H, $d$, $J$ = 3.0 Hz, H-5); one oxymethine proton at $d_{H}$ 3.83 (1H, $t$, $J$ = 5.1 Hz, H-3); one aromatic methyl group at $d_{H}$ 2.15 (3H, $s$, H-11) and one system of two diastereotopic protons appearing as a doublet of doublet at $d_{H}$ 2.90 (1H, $dd$, $J$ = 17.1, 4.8 Hz, H-4β) and 2.70 (1H, $dd$, $J$ = 17.1, 5.1 Hz, H-4α). These data are characteristic of a chromanol skeleton, with a tetra substituted aromatic ring (Sabine et al. 1999). The same spectrum displays signals of three olefinic methines at $d_{H}$ 5.12 (3H, $m$, H-3′, 7′, 11′); six methylenes groups at $d_{H}$ 2.16 (2H, $m$, H-2′), 2.09 (2H, $m$, H-6′), 2.07 (2H, $m$, H-10′), 2.01 (2H, $m$, H-5′), 1.99 (2H, $m$, H-9′) and 1.56 (2H, $m$, H-1′) and five methyl groups at $d_{H}$ 1.71 (3H, $s$, H-13′), 1.63 (3H, $s$, H-14′), 1.61 (3H, $s$, H-16′), 1.60 (3H, $s$, H-15′) and 1.34 (3H, $s$, H-17′). The broad band decoupled $^{13}$C NMR spectrum revealed 27 carbon signals, which were sorted by DEPT and HMBC into six aromatic carbons at $d_{C}$ 116.2 (C-7), 113.4 (C-5), 148.7 (C-6), 144.5 (C-9), 119.2 (C-10) and 127.5 (C-8); six olefinic carbons at $d_{C}$ 135.6 (C-4′), 135.0 (C-8′), 131.2 (C-12′), 124.1 (C-7′), 124.4 (C -11′) and 123.8 (C -3′); two oxygenated carbons at $d_{C}$ 77.9 (C-2) and 68.7 (C-3); Seven methylene groups at $d_{C}$ 31.4 (C-4), 39.7 (C-5′), 39.6 (C-9′), 36.8 (C-1′), 26.7 (C-6′), 26.5 (C -10′) and 21.7 (C-2′) and six methyl carbons at $d_{C}$ 25.7 (C-13′), 17.7 (C-14′), 16.0 (C-16′), 19.3 (C-17′), 16.1 (C-11) and 15.9 (C-15′). The HMBC and COSY spectra enabled to build a phytol fragment (Figures S15 and S16), that was linked at C-2 carbon of the chromanol skeleton. In fact, the HMBC spectrum exhibited correlations (Figure S15) between H-5 with C-9, C-4, C-6 and C-7; between H-7 with C-9, C-
11, C-6 and C-5; between H-4 with C-9, C-8 and C-7; while significant COSY 1H-1H correlations (Figure S16) were observed between H-3/H-4, H-7/H-5, H-3′/H-2′, H-7′/H-6′ and H-11′/H-10′. Compound 1 is different from 8-methyltocotrienol (Ohnmaclt et al. 2008; De Mesquita et al. 2011) at C-3 by the substitution of the methylene carbon by an oxymethine carbon. The values of the coupling constant between H-3 and H-4 (J_{1,2} = 4.8, 5.1 Hz) leads to conclude that C3-OH and the methyl group at C-2 are in pseudoequatorial orientation. The determination of the relative configuration of C3-OH based on NOESY is difficult because the cyclohexane moiety adopted a half chair configuration (Kuroda et al. 2018), and therefore, the H-3 proton correlated with H-4 (α, β, H-17′ and H-1′), while the H-4 (α, β) correlated with the H-17′ (Figure S17). According to the biogenesisc of vitamins E (Sabine et al. 1999), and the structural similarity with thunbergols A (Seo et al. 2006), the relative configurations of the asymmetric centers of compound 1 were defined as 2R*, 3R*. Hence, compound 1 was elucidated as (2R*,3R*)-3α-hydroxy-δ-tocotrienol.

Compound 1a (6-O-allyl-3α-hydroxy-δ-tocotrienol) was obtained as colorless oil from the allylation reaction of 3-hydroxy-δ-tocotrienol (1), with [α]_{D}^{20} = -3 (C = 1.55 mg/mL, CH_{2}Cl_{2}). The molecular formula of 1a was determined as C_{30}H_{42}O_{3} from (+)-HREIMS where an [M]^{+} ion peak was observed at m/z 452.3280 (calcd 452.32850). Compound 1a has 40 mass units higher than compound 1, corresponding to one allyl substituent. The 1H NMR spectrum of 1a exhibited in addition to the signals of 3-hydroxy-δ-tocotrienol (1), typical signals of an allyl fragment at δ_{H} 5.90 (1H, ddt, J = 17.3, 10.4, 5.1 Hz, H-2′); 5.24 (1H, dq, J = 17.3, 1.8 Hz, H-3a′); 5.06 (1H, m, H-3b′) and at δ_{H} 4.31 (2H, dt, J = 5.1, 1.6 Hz, H-1′′). The 13C NMR spectrum corroborates the 1H NMR with the presence of additional signals at δ_{C} 134.8 (C-2′′), 116.2 (C-3′′) and 69.1 (C-1′′). The allyl fragment was linked to C-6 (δ_{C} 152.1) according to the HMBC correlations from the protons at δ_{H} 4.31 (H-1′′) and the carbons C-6.

Compounds 1b (6-O-prenyl-3α-hydroxy-δ-tocotrienol) and 1c (6-O,5-C-diprenyl-3α-hydroxy-δ-tocotrienol) were all obtained as colorless oils from the prenylation reaction of 3-hydroxy-δ-tocotrienol (1), with [α]_{D}^{20} = -3.2 (C = 1.0 mg/mL, CH_{2}Cl_{2}), and [α]_{D}^{20} = -7.5 (C = 1.0 mg/mL, CH_{2}Cl_{2}), respectively. The molecular formula of 1b and 1c were respectively determined as C_{32}H_{48}O_{3}Na^{+} and C_{37}H_{56}O_{3}Na^{+}, from the (+)-HRESIMS, [M + Na]^{+} and (+)-HRESIMS, [M + Na]^{+}; where ions peaks were found at m/z 503.3503 and 571.4120 (calcd: 503.34957 and 571.41217). Compound 1b and 1c have respectively 68 and 136 mass units higher than compound 1, corresponding to one and two prenyl fragments. The 1H NMR spectra of 1b and 1c exhibited in addition to the signals of 3α-hydroxy-δ-tocotrienol (1), typical signals of one and two 3,3-dimethylallyl groups at [δ_{H} 5.30 (1H, ddt, J = 6.6, 5.1, 1.4 Hz, H-2′); 4.30 (2H, d, J = 6.5 Hz, H-1′′); 1.52 (3H, s, H-4′′); 1.05 (3H, s, H-5′′)] and [δ_{H} 5.40 (1H, ddt, J = 8.1, 5.1, 1.4 Hz, H-2′′); 4.96 (1H, m, H-2′′′); 4.35 (2H, d, J = 6.6 Hz, H-1′′′); 3.21 (2H, d, J = 6.8 Hz, H-1′′′′); 1.70 (6H, m, H-4′′ and H-4′′′′); 1.68 (3H, m, H-5′′′′); 1.64 (3H, m, H-5′′′′′)] respectively. The prenyl fragment were linked to C-6 (δ_{C} 152.9) according to the HMBC correlations from the protons at δ_{H} 4.30 (H-1′′) and the carbons C-6, for compound 1b, and following the HMBC correlations from the protons at δ_{H} 4.35/3.21 (H-1′′/H-1′′′′) and the carbons C-6 (149.9)/C-5 (127.0) for compound 1c. Compound 1c results from the mesomeric effect on the potassium phenate intermediate (Scheme 1).
Compound 1 and its derivatives were evaluated for their antioxidant activity using DPPH scavenging method. It was about a hundred times more active than the reference trolox (26 μM), with IC_{50} value of 0.25 μM, while the semisynthetic derivatives (1a-1c) were inactive. The inactivity of derivatives was in accordance with the fact that the free hydroxyl group is responsible of the activity. There are comparatively very few studies in the literature related to the antioxidant activities of tocotrienols in general and δ-tocotrienols in particular. It has been stated that generally γ-tocotrienol has a higher antioxidant effect than α-tocotrienol, and tocotrienols may be better antioxidants than their corresponding tocopherols in certain oils and fats systems (Seppanen et al. 2010).

The cytotoxic activities of compound 1, and its semisynthetic derivatives (1a-1c), were also determined in cell-based cytotoxicity assays using human cervix carcinoma KB-3-1 cells line. Only compound 1 exhibited significant cytotoxic activities with IC_{50} values of 97 μM, while the semisynthetic derivatives (1a-1c) were inactive, compared to the reference drug griseofulvin (17-21 μM). It’s evidence that the free hydroxyl group on the aromatic ring is the main functional group responsible for the activity. These results corroborated some previous data on tocotrienols (Ahsan et al. 2014).

3. Experimental

3.1. General experiment procedures

The ^1^H and ^13^C NMR spectra were recorded on Bruker DRX spectrometers at 500 MHz and 125 MHz respectively. HRESIMS spectra were recorded on a Synapt G2Si -Q-IMS-TOF-mass spectrometer. Column chromatography (open column) was performed with silica gel Merck 60 (0.063-0.200 mm) and percolated aluminum backed silica gel 60 F254 sheets were used for TLC. Size exclusion column chromatography was performed.
using Sephadex LH-20. The TLC spots were visualized under UV light (254 and 365 nm); \( \text{H}_2\text{SO}_4\text{conc.} \) (10\%) was used as spraying reagents.

### 3.2. Plant material and identification

The seeds of *Allophylus africanus* were collected in October 2017 in Bangoua, West Region of Cameroon and identified at the Cameroon National Herbarium, Yaoundé, by Victor Nana, with voucher specimen No 20561/SRF/HNC.

### 3.3. Extraction and isolation

After air-drying and grinding, 1.5 kg of powdered seeds of *Allophylus africanus* were obtained and macerated in methanol (8 L) twice for 72 hours at room temperature, to give 233.0 g of the MeOH crude extract after evaporation of solvent under reduced pressure. 200.0 g of MeOH crude extract was fractionated by CC on silica gel with \( \text{CH}_2\text{Cl}_2/\text{MeOH} \) solvent mixtures of increasing polarities: \( \text{CH}_2\text{Cl}_2/\text{MeOH} \) (97.5:2.5), \( \text{CH}_2\text{Cl}_2/\text{MeOH} \) (95:5), \( \text{CH}_2\text{Cl}_2/\text{MeOH} \) (90:10), \( \text{CH}_2\text{Cl}_2/\text{MeOH} \) (85:15) and \( \text{CH}_2\text{Cl}_2/\text{MeOH} \) (70:30) to give five major fractions indexed A (33.0 g), B (20.0 g), C (10.0 g), D (20.0 g) and E (65.0 g), respectively. Fraction A (33.0 g) was chromatographed on a silica gel column with the mixtures of Hex/\( \text{CH}_2\text{Cl}_2 \) (1:1) and \( \text{CH}_2\text{Cl}_2/\text{MeOH} \) (ratio) of increasing polarity to give after grouping, three major sub-fractions (A1-A3). The sub-fractions A1 and A2 were respectively distributed under the bases of their TLC profiles into three sub-fractions (A1a-A1c) and two sub-fractions (A2a-A2b). Compound 5 (10.0 mg), compound 8 (50.0 mg) and compound 6 (12.0 mg) were obtained from subfractions A1a, A1b and A2a respectively. Compounds 7 (25.0 mg), 4 (6.0 mg) and 1 (120.0 mg) were directly obtained from the sub-fractions A1c, A2b and A3 respectively. Fraction E (65.0 g) was purified by silica gel CC using AcOEt/MeOH (100:00-85:15) as eluent to give three major sub-fractions (E1-E3). Compound 3 (130.0 mg) was directly obtained from sub-fraction E1 while compounds 2 (7.0 mg) and 9 (150.0 mg) were respectively obtained after purification of the sub-fraction E2 on Sephadex LH-20 CC with MeOH as eluent.

### 3.4. Allylation and prenylation reactions

#### 3.4.1. Allylation of 3α-hydroxy-δ-tocotrienol

25.0 mg of 3α-hydroxy-δ-tocotrienol were dissolved in 10.0 mL of acetone. Then, 120.0 mg of potassium carbonate (\( \text{K}_2\text{CO}_3 \)) and 0.2 mL of allyl bromide were respectively added to this mixture. The reaction medium was refluxed at 70 °C for 9 h (Barron et al. 1996). Compounds 1a (yield 55\%) was obtained after purification of the reaction product on silica gel CC with petrol ether/\( \text{CH}_2\text{Cl}_2 \) (95:5) as eluent.

#### 3.4.2. Prenylation of 3α-hydroxy-δ-tocotrienol

28.0 mg of 3α-hydroxy-δ-tocotrienol were dissolved in 10.0 mL of acetone; then, 170.0 mg of potassium carbonate (\( \text{K}_2\text{CO}_3 \)) and 0.35 mL of dimethylallyl bromide were respectively added. The reaction medium was refluxed at 85 °C for 18 h (Barron et al.
1996). Compounds 1b (yield 37%) and 1c (yield 27%) were obtained after purification of the reaction products on silica gel CC with petrol ether/CH₂Cl₂ (95:5) as eluent.

3α-hydroxy-δ-tocotrienol (1): white amorphous solid; [α]D²⁰ = −1.3 (C = 1.22 mg/mL, CH₂Cl₂); UV (nm): 320; (+)-HREIMS: m/z 413.3050 [M + H]⁺ (calcd 413.30502, for C₂₇H₄₄O₃Na⁺). ¹H NMR (CDCl₃, 500 MHz), δH (ppm): 6.52 (1H, d, J = 3.0 Hz, H-7); 6.35 (1H, d, J = 3.0 Hz, H-5); 5.12 (3H, m, H-3'/7'/11'); 3.83 (1H, t, H-3); 2.90 (1H, dd, J = 17.1, 4.8 Hz, H-4a); 2.70 (1H, dd, J = 17.1, 5.1 Hz, H-4b); 2.16 (2H, m, H-2'); 2.15 (3H, s, H-11); 2.09 (2H, m, H-6'); 2.07 (2H, m, H-10'); 2.01 (2H, m, H-5'); 1.99 (2H, m, H-9'); 1.71 (3H, s, H-13'); 1.63 (3H, s, H-14'); 1.61 (3H, s, H-16'); 1.60 (3H, s, H-15'); 1.34 (3H, s, H-17'); 1.56 (2H, m, H-1'). ¹³C NMR (CDCl₃, 125 MHz), δC (ppm): 148.7 (C-6), 144.5 (C-9), 135.6 (C-4'), 135.0 (C-8'), 131.2 (C-12'), 127.5 (C-8), 124.1 (C-7'), 124.4 (C-11'), 123.8 (C-3'), 119.2 (C-10), 116.2 (C-7), 113.4 (C-5'), 77.9 (C-2), 68.7 (C-3), 39.7 (C-5'), 39.6 (C-9'), 36.8 (C-1'), 31.4 (C-4), 26.7 (C-6'), 26.5 (C -10'), 25.7 (C-13'), 21.7 (C-2'), 19.3 (C-17'), 17.7 (C-14'), 16.1 (C-11), 16.0 (C-16'), 15.9 (C-15').

6-O-allyl-3α-hydroxy-δ-tocotrienol (1a): colorless oil; [α]D²⁰ = −3.0 (C = 1.55 mg/mL, CHCl₃). (+)-HREIMS: m/z 452.3280 [M]⁺ (calcd 452.3285 for C₃₀H₄₄O₃Na³⁺). ¹H NMR (Acetone-d₆, 500 MHz), δH (ppm): 6.45 (1H, d, J = 3.0 Hz, H-7); 6.34 (1H, d, J = 3.0 Hz, H-5); 5.90 (1H, ddt, J = 17.3, 10.4, 5.1 Hz, H-2'); 5.24 (1H, dq, J = 17.3, 1.8 Hz, H-3'a); 5.06 (1H, m, H-3b'); 4.98 (3H, m, H-3'/7'/11'); 4.31 (2H, dt, J = 5.1, 1.6 Hz, H-1'); 3.71 (1H, dt, J = 8.4, 5.7 Hz, H-3); 2.80 (1H, dd, J = 16.6, 5.7 Hz, H-4a); 2.57 (1H, dd, J = 16.6, 8.4 Hz, H-4b); 2.10 (2H, m, H-2'); 1.99 (3H, s, H-11); 1.92 (4H, m, H-6'/H-10'); 1.85 (4H, m, H-5'/H-9'); 1.58 (3H, s, H-1'); 1.52 (3H, s, H-13'); 1.49 (3H, s, H-16'); 1.46 (6H, s, H-15'/H-17'); 1.05 (2H, m, H-14'). ¹³C NMR (Acetone-d₆, 125 MHz), δC (ppm): 152.1 (C-6), 145.5 (C-9), 134.9 (C-4'), 134.8 (C-8'), 134.8 (C-2'), 130.6 (C-12'), 126.7 (C-8), 125.0 (C -11'), 124.6 (C-7'), 124.5 (C -3'), 120.7 (C-10), 116.2 (C-3'), 115.9 (C-7), 112.6 (C-5), 78.3 (C-2), 69.1 (C-1'), 68.0 (C-3), 39.9 (C-9'), 39.8 (C-5'), 38.4 (C-1'), 32.1 (C-4), 26.9 (C-6'), 26.7 (C-10'), 25.3 (C-13'), 21.7 (C-2'), 17.5 (C-14'), 17.2 (C-17'), 15.9 (C-16'), 15.5 (C-15'), 15.4 (C-11).

6-O-propenyl-3α-hydroxy-δ-tocotrienol (1b): colorless oil; [α]D²⁰ = −3.2 (C = 1.0 mg/mL, CHCl₃). (+)-HREIMS: m/z 503.3503 [M + Na]⁺ (calcd 503.34957 for C₃₀H₄₄O₃Na⁺). ¹H NMR (Acetone-d₆, 500 MHz), δH (ppm): 6.43 (1H, d, J = 3.0 Hz, H-7); 6.33 (1H, d, J = 3.0 Hz, H-5); 5.30 (1H, ddt, J = 6.6, 5.1, 1.4 Hz, H-2'); 5.07 (1H, m, H-11'); 4.98 (2H, m, H-3'/7'/11'); 4.30 (2H, dt, J = 5.1, 1.6 Hz, H-1'); 3.71 (1H, dt, J = 8.5, 5.6 Hz, H-3); 2.80 (1H, dd, J = 16.6, 5.7 Hz, H-4a); 2.57 (1H, dd, J = 16.6, 8.5 Hz, H-4b); 2.10 (2H, m, H-2'); 1.99 (3H, s, H-11); 1.96 (2H, m, H-10'); 1.92 (2H, m, H-5'); 1.88 (2H, m, H-9'); 1.62 (3H, s, H-13'); 1.59 (2H, s, H-1'); 1.58 (3H, m, H-14'); 1.52 (3H, s, H-4'); 1.49 (3H, s, H-15'); 1.46 (6H, m, H-16'/H-17'); 1.05 (3H, s, H-5'). ¹³C NMR (Acetone-d₆, 125 MHz), δC (ppm): 151.9 (C-6), 144.9 (C-9), 136.0 (C-3'), 135.9 (C-4'), 135.8 (C-8'), 130.6 (C-12'), 126.1 (C-8), 124.7 (C -11'), 124.2 (C-7'), 124.1 (C -3'), 120.8 (C-2'), 120.2 (C-10), 115.5 (C-7), 112.1 (C-5), 77.8 (C-2), 67.7 (C-3), 64.7 (C-1'), 39.5 (C-5'/C-9'), 38.0 (C-1'), 31.7 (C-4), 26.5 (C-6'), 26.3 (C -10'), 24.9 (C-4'), 24.8 (C-13'), 21.3 (C-2'), 17.2 (C-14'), 17.1 (C-5'), 16.8 (C-17'), 15.5 (C-11), 15.1 (C-16'), 15.0 (C-15').

6-O,5-C-diprenyl-3α-hydroxy-δ-tocotrienol (1c): colorless oil; [α]D²⁰ = −7.5 (C = 1.0 mg/mL, CHCl₃). (+)-HREIMS: m/z 571.4120 [M + Na]⁺ (calcd 571.41217 for C₃₀H₄₄O₃Na⁺). ¹H NMR (CDCl₃, 500 MHz), δH (ppm): 6.55 (1H, s, H-7); 5.40 (1H, ddt, J = 8.1, 5.1, 1.4 Hz, H-2'); 5.03 (3H, m, H-11'/H-3'/7'); 4.96 (1H, m, H-2''); 4.35 (2H, d,
$J = 6.6$ Hz, H-1'); 3.77 (1H, m, H-3); 3.21 (2H, d, $J = 6.8$ Hz, H-1'); 2.87 (1H, dd, $J = 16.9$, 5.3 Hz, H-4a); 2.62 (1H, dd, $J = 16.9$, 5.9 Hz, H-4b); 2.10 (3H, s, H-11); 2.08 (2H, m, H-2'); 1.98 (4H, m, H-5'/H-9'); 1.89 (2H, m, H-6'/10'); 1.70 (6H, m, H-4'/H-4''); 1.68 (3H, m, H-5'); 1.64 (3H, m, H-5'''); 1.61 (3H, s, H-13'); 1.53 (5H, m, H-1'/H-14'); 1.50 (6H, s, H-15'/H-16'). 13C NMR (CDCl3, 125 MHz), $\delta_C$ (ppm): 149.9 (C-6), 144.8 (C-9), 136.6 (C-30'), 135.5 (C-40), 135.0 (C-80), 127.0 (C-5), 124.3 (C –11'), 124.1 (C-7'), 124.0 (C-3'/C-8), 122.5 (C-2'''), 120.8 (C-2'), 118.4 (C-10), 114.3 (C-7), 77.1 (C-2), 68.8 (C-3), 66.4 (C-1'), 39.7 (C-5'/C-9'), 36.9 (C-1'), 29.5 (C-4), 26.7 (C-6'), 26.5 (C –10'), 25.8 (C-13'), 25.7 (C-4'/C-4''), 24.9 (C-1'''), 21.6 (C-2'), 18.9 (C-17'), 18.1 (C-5'''), 17.9 (C-5''), 17.7 (C-14'), 16.2 (C-11), 16.0 (C-16'), 15.8 (C-15').

3.5. Antioxidant assay

The DPPH (1,1-diphenyl-2-picrylhydrazyl) assay was used to evaluate the free radical scavenging activity of the compounds (Sellem et al. 2016). Briefly, the compounds were dissolved in 10% DMSO and diluted at different concentrations of 250.0 to 1.0 µg/mL. Then, 500.0 µL of a 4% (w/v) solution of DPPH radical in methanol was mixed with 500.0 µL of samples under investigation. The mixture was incubated for 30 min in the dark at room temperature. The scavenging capacity was determined spectrophotometrically by monitoring the decrease in absorbance at 517 nm against a blank. The percentage of antioxidant activity was calculated

$$\text{As antioxidant activity} \% = \left[ \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \right] \times 100.$$

BHA (Butylhydroxyanisol) and BHT (Butylatedhydroxytoluene) (Sigma, USA) were used as positive controls. $A_{\text{sample}}$ was the absorbance of the sample and $A_{\text{control}}$ was the absorbance of the blank.

3.6. Cytotoxicity assay

Cytotoxic activity screening of the isolates was done as described in previous reports (Sammet et al. 2010; Awantu et al. 2011). The KB-3-1 cell was cultivated as a monolayer in DMEM (Dulbecco’s modified Eagle medium) with glucose (4.5 g.L$^{-1}$), L-glutamine, sodium pyruvate and phenol red, supplemented with 10% (KB-3-1) foetal bovine serum (FBS). The cells were maintained at 37 °C and 5.3% CO$_2$-humidified air. The day before the test, the cells (70% confluence) were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) solution (0.05%; 0.02% PBS) and placed in sterile 96-well plates in a density of 10 000 cells in 100 µL medium per well. The dilution series of the compounds were prepared from stock solutions in DMSO of concentrations of 1 mM or 10.0 mM. The stock solutions were diluted with culture medium 10% FBS (KB-3-1]) at least 50 times. Some culture medium was added to the wells to adjust the volume of the wells to the wanted dilution factor. The dilution prepared from stock solution was added to the wells. Each concentration was tested in six replicates. Dilution series were prepared by pipetting liquid from well to well. The control contained the same concentration of DMSO as the first dilution. After incubation for 72 h at 37 °C and 5.3% CO$_2$-humidified air, 30.0 µL of an aqueous resazurin solution (175.0 µM) were added to each well. The cells were incubated at the same conditions for 6 h.
Subsequently, the fluorescence was measured. The excitation was affected at a wavelength of 530 nm, whereas the emission was recorded at a wavelength of 588 nm. The \( IC_{50} \) values were calculated as a sigmoidal dose response curve using GRAPHPAD PRISM 4.03. The \( IC_{50} \) values equal the drug concentrations, at which vitality is 50%. Griseofulvin was used as reference.

### 4. Conclusions

The seeds of *A. africanus* were investigated for their chemical composition, antioxidant and cytotoxicity activities in this study for the first time. The chemical investigation led to the isolation of nine compounds, including a new tocotrienol namely, 3\( \alpha \)-hydroxy-\( \delta \)-tocotrienol (1). The allylation and the prenylation of compounds 1 led to the three new semi-synthesis derivative 1a and 1b-1c respectively namely, 6-O-allyl-3\( \alpha \)-hydroxy-\( \delta \)-tocotrienol (1a), 6-O-prenyl-3\( \alpha \)-hydroxy-\( \delta \)-tocotrienol (1b) and 6-O-5-C-diprenyl-3\( \alpha \)-hydroxy-\( \delta \)-tocotrienol (1c). DPPH scavenging method and human cervix carcinoma KB-3-1 cells line assays were performed to assess the antioxidant and cytotoxicity of compound 1 and its derivatives. Compound 1 was about a hundred times more active than the reference trolox (26 \( \mu \)M) with \( IC_{50} \) value of 0.25 \( \mu \)M regarding the antioxidant activity, and exhibited significant cytotoxic activities with \( IC_{50} \) values of 97 \( \mu \)M compared to the reference drug griseofulvin (17-21 \( \mu \)M), while the semisynthetic derivatives (1a-1c) were inactive. These results corroborate some data on certain \( \delta \)-tocotrienol derivatives, with regard to the antioxidant and the cytotoxicity activities (Beretta et al. 2018; Popova et al. 2020).

### Notes

The authors declare no competing financial interest.

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