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Antileishmanial and cytotoxic activities of a new limonoid and a new phenyl alkene from the stem bark of *Trichilia gilgiana* (Meliaceae)

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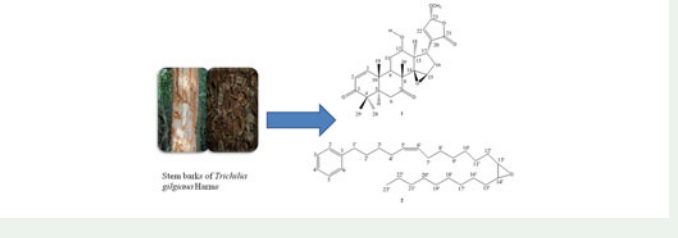
ABSTRACT

One new limonoid, trigilgianin (**1**), one new phenyl alkene, epoxy gilgialkene (**2**), together with five known compounds: scopoletin (**3**), sitosteryl-6′-*O*-undecanoate-β-D-glucoside (**4**), sitosteryl-*O*-β-D-glucopyranoside (**5**), cinchonain A (**6**) and cinchonain B (**7**) were isolated from the stem bark of *Trichilia gilgiana* Harms. (Meliaceae). All compounds were isolated for the first time from this species. The structures were elucidated on the basis of spectral studies and by comparison of these data with those from the literature. Compounds **1**, **2**, **3**, **6** and **7** were tested for *in vitro* antileishmanial activity against visceral leishmaniasis parasite *Leishmania donovani* and cytotoxicity against macrophage RAW 264.7 cell line. Compounds **1** and **3** showed the highest antileishmanial activity (IC_{50} values of 6.044 and 6.804 µg/mL, respectively) with low cytotoxicity (CC_{50} values of >200 and 47.47 µg/mL, respectively), while compound **2** was moderately active on *L. donovani* promastigotes (IC_{50} 56.81 µg/mL).



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1. Introduction

Leishmaniasis is a major public health problem in 98 endemic countries where it is responsible for approximately 2–4 million new cases and around 70,000 deaths per year (Armeli Minicante et al. 2016). This neglected tropical disease is caused by parasites belonging to the kinetoplastidae family and belonging to the zoonotic and vector-borne disease. This complexe disease comprises a variety of manifestations including cutaneous leishmaniasis that affect macrophages resident in the skin, and visceral leishmaniasis, the deadliest and most severe form affecting mononuclear phagocyte system cells of liver, spleen, bone marrow, lymph nodes and intestine (Steverding 2017). Despite the fact that available drugs display several side effects and are affected by parasite resistance, they still remain the first option for the treatment, and therefore this highlights the urgent need for new and improved therapy. For centuries, plants have been used as a rich source of novel compounds for the treatment of several diseases.

Trichilia gilgiana Harms is one of the largest trees in the Meliaceae family, mainly distributed in the South West Region of Cameroon (Tatcham et al. 2015). The stem bark of this plant is used in traditional medicine to treat typhoid fevers (Tatcham et al. 2015), abdominal, and fever pains (Louppe 2008). Previous phytochemical investigations of some *Trichilia* species led to the isolation of limonoids (Sabrina et al. 2004; Tsamo et al. 2013; Liu et al. 2017; Nangmo et al. 2018), cycloartanes (Tsamo et al. 2013), steroids (Pupo et al. 1997), coumarins (Tsamo et al. 2018) and flavalignans (Moacir et al. 2002). To the best of our knownledge, no previous phytochemical study was reported on the stem bark of *T. gilgiana*. In the continuation of our effort in the search for bioactive compounds from Cameroonian medicinal plants (Kopa et al. 2014; 2016), we investigated the constituents of stem bark of *T. gilgiana* as well as the evaluation of antileishmanial and cytotoxicity activities of compounds **1**, **2**, **3**, **6** and **7** against visceral leishmaniasis parasites, *L. donovani* promastigotes *in vitro* and macrophage Raw 264.7 cell line, respectively.

2. Results and discussion

2.1. Structure elucidation of new compounds

The ethyl acetate soluble fraction of the stem bark of *T. gilgiana* was subjected to silica gel and Sephadex LH-20 column chromatography, followed by preparative TLC to obtain a new limonoid: trigilgianin (**1**), a new phenyl alkene: epoxygilgialkene (**2**) along with five known compounds scopoletin (**3**) (Tsamo et al. 2013), sitosteryl-6′-*O*-undecanoate-β-D-glucoside (**4**) (Mahmoud et al. 2009), sitosteryl-β-D-glucopyranoside (**5**) (Tsamo et al. 2013), cinchonain A (**6**) and cinchonain B (**7**) (Moacir et al. 2002) (Figure 1). The structures of the known compounds were identified by comparison of their spectroscopic data with those reported in the literature.

Compound **1** was obtained as a white powder from a mixture of *n*-Hex/EtOAc (90:10, v/v). It reacted positively both with Liebermann-Burchard (red color) and Erhlich (pink color) tests suggesting its limonoidic nature. Its molecular formula, $C_{27}H_{34}O_7$, was deduced from NMR data (Table S1 and Figures S4–S10) and (-) HR-ESI-

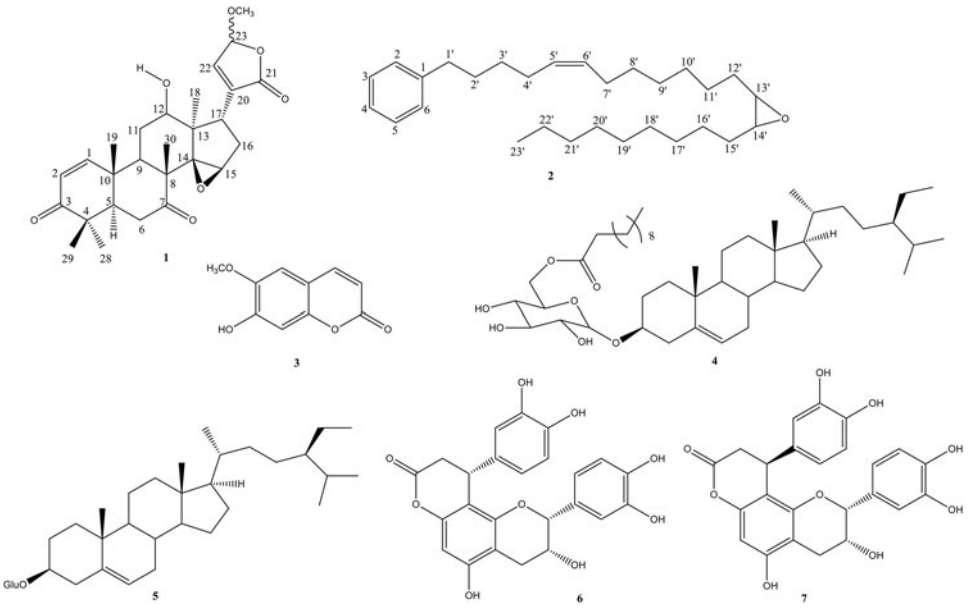

Figure 1. Chemical structures of compounds (**1**–**7**) isolated from *T. gilgiana*.

MS (Figure S11), from which a molecular ion adduct peak was obtained at *m/z* 515. 2223 $[M + HCOOH\text{-}H]^-$ (calcd. for $C_{28}H_{35}O_9$ 515.2287), indicating 11 degrees of unsaturation. The IR spectrum showed the presence of free hydroxyl group (3400 cm^{-1}), carbonyl of lactone group (1743 cm^{-1}), and an α,β-unsaturated ketone moieties (1666, 1720 cm^{-1}). The UV spectrum indicated the presence of an α,β-unsaturated carbonyl group (196, 211 and 276 nm). The structure of **1** was assigned by analyses of 1 D (^1H, ^{13}C) and 2 D (HSQC, HMBC, ^1H-^1H-COSY, and NOESY) NMR and MS data. The ^1H-NMR spectrum (Table S1) showed signals of five tertiary methyl groups at δ_H 1.37 (s, H-19), 1.24 (s, H-30), 1.18 (s, H-29), 1.16 (s, H-28), 1.14 (s, H-18), three oxygenated methine protons at δ_H 3.93 (s, H-15), 5.44 (d, $J = 10.2$ Hz, H-12), 6.19 (d, $J = 13.9$ Hz, H-23), one methyl oxygenated at δ_H 3.93 (s, 23-OCH_3), two olefinic protons at δ_H 5.95 (d, $J = 10.8$ Hz, H-2), 7.12 (d, $J = 10.8$ Hz, H-1), and one additional olefinic proton at δ_H 7.35 (d, $J = 10.8$ Hz, H-22). The ^{13}C NMR spectrum (Table S1) of compound **1** displayed 27 carbon resonances, which were further sorted by APT and HSQC experiments as six methyls, three methylenes, nine methines (three oxygenated and three olefinic), and nine quaternary carbons (two carbonyls, one oxygenated and one olefinic). The ^1H- and ^{13}C –NMR spectra (Figures S4–S5) exhibited resonances assignable to three carbonyl groups including two keto carbonyls at δ_C 203.0 (C-3), 208.6 (C-7), and one carbonyl of a lactone group at δ_C 165.8 (C-21), four olefinic carbons at δ_C 155.7 (C-1), 126.8 (C-2), 150.8 (C-22), 133.8 (C-20). These data indicated that compound **1** is a tetranotriterpenoid with similar skeleton like cedrelone-type limonoids, possessing the identical rings A-D compare to 23-hydroxycedrelonelide (Zhang et al. 2012); limonoids with similar skeleton which were also reported in *Trichilia americana* (Ji et al. 2015). Olefinic protons centered at δ_H 7.12 (d, $J = 10.8$ Hz, H-1), δ_H 5.95 (d, $J = 10.8$ Hz, H-2) comprise an AX system corresponding to the protons in an α,β-unsaturated carbonyl moiety, which was indicated by the carbon resonances at δ_C 155.7 (C-1), 126.8 (C-2), and 203.0 (C-3),

the locations of which were determined by the HMBC correlations (Figure S1) observed between H_3-28 (δ_H 1.16), H_3-29 (δ_H 1.18) and C-3 (δ_C 203.0) and between H_3-19 (δ_H 1.37) and C-1 (δ_C 155.7). The existence of the second carbonyl group at the C-7 position was revealed by HMBC correlations (Figure S1) observed between H_3-30 (δ_H 1.24) and C-7 (δ_C 208.6), and between H-6 (a,b) (δ_H 2.93, 2.45) and C-7 (δ_C 208.6). Pertinent correlation in the HMBC spectrum (Table S1, Figure S1) between H-12 (δ_H 5.44, d, $J = 10.2$ Hz) and C-20 (δ_C 133.8) indicated the location of the hydroxyl group at C-12. A comparison of the ^{1}H and ^{13}C NMR spectroscopic data of **1** with those of Toonaciliavatarin G from *Toona ciliata* (Zhang et al. 2012) implied that they share a similar skeleton, with a noticeable difference being in the presence of a methoxy-substituted α,β-unsaturated-γ-lactone ring at the C-17 position as observed at δ_H 3.93, 6.19, 7.35 and 53.6, 96.8, 133.8, 150.8, 165.8, instead of furan ring. These results were further confirmed by a ^{1}H-^{13}C HMBC correlation between the protons of the methoxy group (δ_H 3.93) and C-23 (δ_C 53.6) and a ^{1}H-^{1}H COSY correlation from H-22 to H-23 (Figure S9). The HMBC correlations (Figure S1) from H-15 (δ_H 3.93), H-17 (δ_H 3.77), H_3-18 (δ_H 1.14), H_3-30 (δ_H 1.24) and C-14 (δ_C 65.2) suggested the presence of an oxygenated quaternary carbon at C-14 (δ_C 65.2) and the HMBC correlations observed between H-16 (a,b) (δ_H 2.30, 1.36) and C-14 (δ_C 65.2) indicated that the epoxy group should be located between C-14 (δ_C 65.2) and C-15 (δ_C 53.5), thus establishing the tetracyclicore of **1**. Using the HMBC experiment, the remaining carbons at δ_C 165.8, 150.8, 133.8, 96.8 could be attributed to C-21, C-22, C-20 and C-23, comprising a rare γ-hydroxybutyrolactone unit (McFarland et al. 2004) with the corresponding ^{1}H-NMR resonances at δ_H 7.35 and 6.19 assigned to (H-22) and (H-23) respectively (Luo et al. 2000).

The relative configuration of **1** was defined by interpretation of NOESY data (Figure S10) and comparison of NMR data with those of 11β-hydroxycedrelone (Luo et al. 2000). The NOESY (Figure S10) cross-peaks of H-12/H-6b, H-12/H-16b, H_3-29/H-6b, H-5/H-6b, H-9/H-6b, H_3-29/H-6b and H_3-18/H-16b indicated that H-9, H-12, Me-18, H-6b, H-16b, Me-29 and the C-17 side chain were α-oriented. Likewise, the NOESY correlations (Figure S10) of H_3-28/H_3-19, H_3-30/H-6a, H_3-30/H-17, and, H_3-30/H-11b indicated that Me-28, Me-30, Me-19, H-11b and H-17 were cofacial and were randomly assigned to be β-oriented. The 14,15-epoxy ring was fixed as β-oriented based on the matching NMR data of the related protons and carbons with those of 11β-hydroxycedrelone (Luo et al. 2000). In addition, the 14,15-oxirane function was also established to be β-orientated according to a NOESY correlation between H-15 and H-18. Compound **1** was thus elucidated as depicted, featuring a 23-methoxy-20(22)-ene-23,21-γ-lactone ring in the side chain. It is trivially name trigilgianin (**1**), isolated and characterised for the first time from this plant.

Compound **2** was obtained as a brown crystal. Its molecular formula was deduced as $C_{29}H_{48}O$ from its pseudo-molecular ion peak at *m/z* 413.2696 $[M+H]^+$ (calcd for $C_{29}H_{48}O$ 413.2705) in an HR-ESI-MS experiment, consistent with six degrees of unsaturation. The ^{1}H NMR spectrum (Figure S9) of **2** exhibited the signals characteristic of an aromatic ring of a phenyl alkene moiety at δ_H 7.26 (brs, H-3 and H-5), 7.17 (dl, $J = 5.8$ Hz, H-2, H-4 and H-6) (Hwang et al. 2013). The ^{1}H NMR spectrum (Figure S13) also showed additional signals at δ_H 5.35 (brs, H-6′), 5.33 (brs, H-5′), 2.76 (brs, Ha-4′), 2.60 (brs, H-1′), 2.30 (m, 2H-12′), 2.04 (brs, 2H-2′), 2.02 (brs, 2H-7′), 1.62 (brs, 2H-13′), 1.59

(brs, H-3′, H-21′), 1.33 (brs, Hb-4′), 1.30 (s, H-22′), 1.29 (brs, H-3′, H-21′), 1.25 (brs, H-8′-12′, H-16′-20′) and 0.88 (brs, H-23′), corresponding to a long alkenyl side chain. The ^{13}C NMR data (Figure S15) in $CDCl_3$ and APT spectra (Figure S15) displayed a total number of twenty nine carbons including six aromatic carbons [δ_C 142.4 (C-1), 128.4 (C-3, C-5), 128.2 (C-2, C-6), 125.5 (C-4)], two ethylenic carbons [δ_C 130.1 (C-6′), and 127.9 (C-5′)], two oxymethine carbons characteristic of an epoxy group [δ_C 56.0 (C-13′, C-14′)], and a carbon due to a fatty acid moiety [δ_C 35.8 (C-1′), 33.9 (C-12′, C-15′), 32.0 (C-2′), 31.5 (C-3′, C-21′), 29.7-29.3 (C-8′-C-10′, C-17′-C-20′), 27.1 (C-7′), 25.6 (C-4′), 24.8 (C-11′, C-16′), 22.6 (C-22′) and 14.1 (C-23′). The HMBC correlations from the protons at δ_H 2.60 (H-1) to the carbons at δ_C 142.4 (C-1), 128.2 (C-2, C-6), 31.5 (C-3′) confirmed that the mono-substitute benzene moiety was located at C-1 (Table S3). The position of double bond on the alkenyl side chain was determined using the HMBC experiment (Figure S12) and the HR-ESI-MS fragmentation patterns (Figure S20). In the HMBC spectrum, a correlation was observed between the proton at δ_H 5.33 (H-5′) and the carbon at δ_C 27.1 (C-7′) and the proton at δ_H 2.02 (H-7′) and the carbon at δ_C 127.9 (C-5′). The ESI-MS (Figure S19) exhibited and ion-fragment at *m/z* 159 [M-$C_{17}H_{33}O$], corresponding to the loss of a phenyl hexyl group, which determined the position of the double bond. Furthermore, the ^{1}H and ^{13}C NMR spectra indicated two olefinic proton signals at δ_H 5.35 (brs, H-6′)/δ_C 130.1 and 5.33 (brs, H-5′)/δ_C 127.9, assignable to the presence of one double bond with a cis (*Z*) configuration. This was evidenced by the chemical shifts of the methylene carbons next to the olefinic carbons at δ_C 27.0 (C-7′) and 25.6 (C-4′) in **2** (Kopa et al. 2016). The position of the epoxyde group was determined by the presence of fragment ions peaks at *m/z* 243 [M-$C_{11}H_{21}O$] and *m/z* 285 [M-C_9H_{19}] in the ESI spectrum. Based on the NMR data above, the structure of **2** was assigned as (*Z*)-2-nonyl-3-(12-phenyldodec-en-1yl) oxirane, trivially name epoxygilgialkene.

2.2. Antileishmanial and cytotoxic activities

Antileishmanial activities of compounds **1**, **2**, **3**, **6** and **7** were evaluated *in vitro* on *L. donovani* promastigotes. Cytotoxicity was determined on macrophages Raw 264.7 cells line in culture. The antileishmanial and cytotoxicity activities are presented in Table S2 as inhibitory concentrations (IC_{50}) and cytotoxic concentration (CC_{50}), respectively. As shown in Table S2, compounds **1** and **3** exhibited the highest antileishmanial activities with IC_{50} values of 6.044 and 6.804 μg/mL, respectively). In addition, the cytotoxicity against RAW cells was >200 and 47.47 μg/mL, respectively, indicating that compound **1** has a high selectivity index of >33.09. Compound **2** was moderately active on *L. donovani* promastigote with an IC_{50} value of 56.81 μg/mL, without sign of cytotoxicity on macrophages (CC_{50} value of >200 μg/mL), and the epimeric mixture of compounds **6** and **7** was inactive. The antileishmanial activities of all the isolated compounds are reported here for the first time. Antileishmanial activities of limonoids have been reported by other authors. Obbo et al. 2013, reported the antileishmanial activities of two limonoids, grandifolione and 7-deacetylkhivorin isolated from *Khaya anthotheca* against *L. donovani* axenic amastigotes with IC_{50} values of 13.31 and 36.71 μg/mL, respectively.

3. Conclusion

Phytochemical investigation of the stem bark of *T. gilgiana* yielded one new limonoid, trigilgianin (**1**), one new phenyl alkene, epoxygilgialkene (**2**) and five known compounds (**3-7**). Compounds **1-7** were isolated for the first time from this plant. In this work, five compounds **1**-**3** and **6**-**7** were screened against *Leishmania donovani* promastigote and also evaluated for their cytotoxicity against Raw cell line. With the highest activity and better selectivity index, the new compound, trigilgianin (**1)** appears to be as candidate against the visceral leishmaniais parasite, - *L. donovani*. Furthermore, the observed antileishmanial activity of trigilgianin (**1**), epoxy gilgialkene (**2**) and scopoletin (**3**) from the stem bark of *T. gilgiana* confirms the ethnomedicinal potential of this plant and could justify further studies which include the evaluation of the antileishmanial activity against the intracellular amastigote form of *L. donovani* and modes of actions of isolated compounds.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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