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Cytotoxic aryltetralin lignans and sesquiterpene lactones from the flowers of *Tithonia diversifolia*

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Abstract. *Tithonia diversifolia* (Hemsl.) A. Gray has been utilized traditionally to treat various diseases, including cancer, acne, acute enteritis, and gastritis. The ethyl acetate extract derived from the flowers of this plant demonstrated potent cytotoxic activity against KB cells, exhibiting 96 % inhibition at a concentration of 1 µg/mL. As a result, phytochemical investigation of the flower extract of *Tithonia diversifolia* led to the isolation of two lignans (**1**–**2**) and six sesquiterpene lactones (**3**–**8**). Their chemical structures were identified as cleiseberharside A (**1**), cleisindoside A (**2**), tirotundin (**3**), 2*β*-methoxydesoxytagitinin B (**4**), tagitinin C (**5**), 1*β*,2*α*-epoxytagitinin C (**6**), tagitinin G (**7**), and tagitinin H (**8**) by the spectroscopic analyses, including ESI-MS, 1D, and 2D NMR spectra, and comparison with the literature data. Notably, compounds **1** and **2** were discovered from the *Tithonia* genus for the first time. Compounds **3-6** displayed significant cytotoxicity against KB, MCF7, HepG2, and A549 cell lines, with IC_{50} values ranging from 0.28 to 3.76 µM. Especially, tagitinin C (**5**) exhibited the strongest cytotoxic effect against all four tested human cancer cell lines (KB, MCF7, HepG2, A549), with IC_{50} values of 0.28, 1.18, 1.38, and 1.15 µM, respectively, comparable to the reference compound ellipticine.

Keywords: *Tithonia diversifolia,* aryltetralin lignan, sesquiterpene lactone, cytotoxicity.

Classification numbers: 1.1.1, 1.1.6, 1.2.1.

1. INTRODUCTION

The genus *Tithonia* (Asteraceae) comprises 64 species distributed in Mexico, Central America, and Cuba, with two species, *T. diversifolia* and *T. rotundifolia,* found in Viet Nam [1, 2]. The genus *Tithonia*, particularly *Tithonia diversifolia*, has attracted considerable scientific interest due to its wide range of ethnomedicinal uses across tropical and subtropical regions [3]. Previous phytochemical studies have revealed that *Tithonia* species are rich sources of biologically active compounds. These investigations have identified various classes of phytochemicals including sesquiterpene lactones, flavonoids, steroids, saponins, tannins, and glycosides [4-8]. Notably, sesquiterpene lactones such as tagitinin C and tagitinin A have been

isolated and reported to possess significant anti-inflammatory, antiplasmodial, antidiabetic, and cytotoxic properties [3]. Methanolic and ethanolic extracts of *Tithonia diversifolia* leaves have also demonstrated antioxidant and antimicrobial activities, further supporting its traditional use in herbal medicine [9-12]. These findings provide a strong basis for continued exploration of *Tithonia* as a potential source of novel therapeutic agents. However, no studies on the chemical constituents from the flowers of *Tithonia diversifolia* (Hemsl.) A. Gray have been reported so far, except for a few studies on its essential oils [13-16]. As part of our project on screening Vietnamese plants for biological activity, an ethyl acetate extract of *T. diversifolia* flowers exhibited strong cytotoxic activity against KB cells, showing 96 % inhibition at 1 μg/mL. Consequently, this plant was selected for further investigation of its chemical constituents and bioactivities. The isolation, structural and biological elucidation of eight compounds including, two aryltetralin-type lignans (**1 - 2**) and six sesquiterpene lactones (**3 - 8**), were described in this paper (Figure 1).

2. MATERIALS AND METHODS

2.1. General experimental procedures

^{1}H-NMR, ^{13}C-NMR and 2D-NMR spectra were acquired using Bruker 600 MHz spectrometer. Chemical shifts were referenced to internal standards as follows: $CDCl_3$ (δ_H 7.26 ppm, δ_C 77.0 ppm), CD_3OD (δ_H 3.30 ppm, δ_C 49.0 ppm) or DMSO-d_6 (δ_H 3.32 ppm, δ_C 39.5 ppm). *J* coupling constants are expressed in Hz. Melting points were recorded on a Boetius instrument. Column chromatography (CC) was performed using silica gel (Kieselgel 60, 400-630 mesh, Merck), Sephadex LH-20 (Sigma), and YMC*GEL (ODS-A, 12 nm S-150 μm, YMC Co., Ltd.). Silica gel 60 F_{254} (Merck) and RP-18 F_{254s} plates (Merck) were used for thin-layer chromatography (TLC). HPLC purification was performed on an Agilent 1260 Infinity II system.

2.2. Plant materials

The flowers of *Tithonia diversifolia* were collected in Ba Vi province, Viet Nam, in November 2023. Botanical identifications were performed by Dr. Nguyen The Cuong at the Institute of Ecology and Biological Resources (VAST). A voucher specimen (VN-2198C) has been deposited at the Institute of Marine Biochemistry (IMBC), VAST.

2.3. Extraction and isolation

The dried flowers of *T. diversifolia* (3.7 kg) were extracted with MeOH (10 L × 4 times) at 40 °C under ultrasonic conditions. These solutions were concentrated *in vacuo* to give 508.0 g of MeOH extract. The MeOH extract was suspended in H_2O (1.2 L) and successively partitioned with *n*-hexane and ethyl acetate. The obtained solutions were concentrated *in vacuo* to yield the corresponding residues: *n*-hexane extract (88.7 g) and ethyl acetate extract (37.5 g). The ethyl acetate extract was chromatographed on a silica gel column eluting with the gradient solvents of *n*-hexane/acetone (0-100 % acetone, *v/v*) to give 10 fractions, EF1-EF10. Fraction EF6 (2.90 g) was chromatographed on a silica gel column eluting with a gradient of *n*-hexane/acetone (10-100% acetone, *v/v*) to yield five subfractions (EF6.1–EF6.5). Subfraction EF6.3 (0.08 g) was chromatographed on a silica gel column eluting with *n*-hexane/EtOAc (3/1, *v/v*) to obtain compound **6** (4 mg) and compound **8** (7 mg). Subfraction EF6.4 (0.3 g) was chromatographed on a silica gel RP-18 column eluting with MeOH/H_2O (4/1, *v/v*), then purified by HPLC eluting with

ACN/H_2O (6/4, *v/v*), at a flow rate of 3 mL/min to obtain compound **7** (5 mg). Fraction EF8 (3.12 g) was chromatographed on a silica gel column eluting with a gradient of *n*-hexane/acetone (10-100% acetone, *v/v*) to yield eight subfractions (EF8.1–EF8.8). Subfraction EF8.7 was chromatographed on a silica gel RP-18 column eluting with MeOH/H_2O (4/1, *v/v*) to give three subfractions (EF8.7.1–EF8.7.3). Purification of subfraction EF8.7.3 by using a semi-preparative reversed-phase C_{18} HPLC column (J'esphere ODS, 250 x 20 mmm, 4µm), with ACN/H_2O (55:45, *v/v*), at a flow rate of 3 mL/min, yielding compounds **5** (5 mg) and **4** (8.0 mg). Subfraction EF8.5 (0.38 g) was further purified by silica gel column chromatography with a CH_2Cl_2/EtOAc gradient (0-100 % EtOAc), followed by a silica gel column chromatography with a mixture of CH_2Cl_2/acetone (9/1, *v/v*) to yield compound **3** (9 mg). Fraction EF9 (6.39 g) was chromatographed on a Sephadex LH-20 column eluting with MeOH (100 %) to give seven subfractions (EF9.1-EF9.7). Subfraction EF9.4 (0.56 g) was chromatographed on a silica gel column, eluting with the gradient mixtures of CH_2Cl_2/MeOH (3-100 % MeOH in CH_2Cl_2, *v/v*) to yield five subfractions (EF9.4.1-EF9.4.5). Subfraction EF9.4.3 was crystallized in CH_2Cl_2/MeOH (9/1, *v/v*) to give compound **2** (10 mg). Subfraction EF9.4.5 (150 mg) was purified by silica gel CC eluting EtOAc/MeOH (95:5, *v/v*) to give compound **1** (12 mg) (Figure 1).

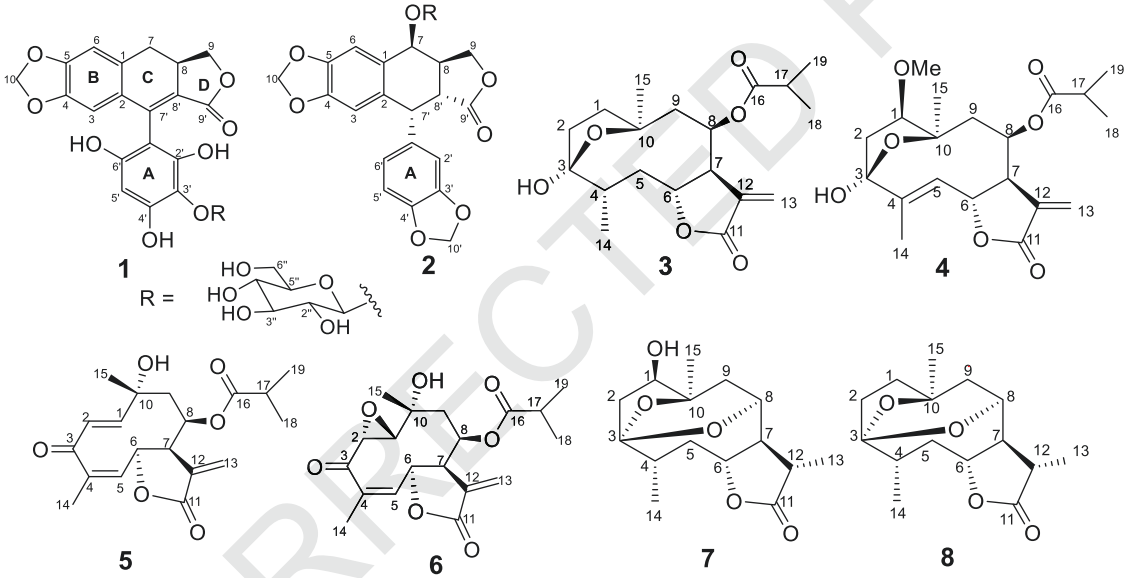

Figure 1. Chemical structures of the isolated compounds **1-8**.

Cleiseberharside A (**1**): white crystal, mp 276–277 °C; $[\alpha]_D^{25}$ -56.1 (*c* 0.1, MeOH); (reference value: $[\alpha]_D^{30}$ –55.89 (*c* 0.11, MeOH [17]); ^{1}H-NMR (DMSO-d_6, 600 MHz) and ^{13}C-NMR (DMSO-d_6, 150 MHz): see Table 1.

Cleisindoside A (**2**): White solid, mp 259-260 °C; $[\alpha]_D^{25}$ -65.4 (*c* 0.1; MeOH); (reference value: $[\alpha]_D^{30}$ -50.0 (*c* 0.3, MeOH) [18]); ^{1}H-NMR (DMSO-d_6, 600 MHz) and ^{13}C-NMR (DMSO-d_6, 150 MHz): see Table 1.

Tirotundin (**3**): Colorless crystal, $[\alpha]_D^{25}$ -71.2 (*c* 0.05, $CHCl_3$) (reference value: $[\alpha]_D^{22}$ -77 (*c* 2.0, $CHCl_3$) [19]); ^{1}H-NMR ($CDCl_3$, 600 MHz) δ_H: 1.04 (3H, d, *J* = 6.6 Hz, CH_3-18), 1.07 (3H, d, *J* = 6.6 Hz, CH_3-19), 1.12 (3H, d, *J* = 7.2 Hz, CH_3-14), 1.16 (1H, m, H-5a), 1.45 (3H, s, CH_3-15), 1.76 (1H, dd, *J* = 12.0, 14.4 Hz, H-9a), 1.85 (1H, m, H-9b), 1.87 (2H, m, H-2), 1.98 (2H, m, H-1b, H-4), 2.13 (1H, m, H-5b), 2.25 (1H, m, H-1a), 2.42 (1H, m, H-17), 4.08 (1H, dd, *J* = 3.0,

6.6 Hz, H-7), 4.56 (1H, ddd, *J* = 1.8, 6.6, 9.6 Hz, H-6), 5.52 (1H, d, *J* = 3.6 Hz, H-13a), 5.54 (1H, m, H-8), 6.26 (1H, d, *J* = 3.6 Hz, H-13b); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ_C: 18.6 (C-19), 18.8 (C-18), 19.2 (C-14), 26.9 (C-15), 34.0 (C-17), 38.0 (C-5), 38.2 (C-2), 38.8 (C-1), 42.1 (C-9), 43.3 (C-4), 47.8 (C-7), 69.6 (C-8), 80.2 (C-10), 81.2 (C-6), 108.8 (C-3), 121.6 (C-13), 137.0 (C-12), 169.4 (C-11), 176.2 (C-16).

2*β*-Methoxydesoxytagitinin B (**4**): Colorless oil, $[\alpha]_D^{25}$ -106.8 (*c* 0.03, $CHCl_3$); (reference value: $[\alpha]_D^{24} - 124$ (*c* 1.3, $CHCl_3$) [20]); ^{1}H-NMR ($CDCl_3$, 600 MHz) δ_H: 1.04 (3H, d, *J* = 6.6 Hz, CH_3-19), 1.06 (3H, d, *J* = 6.6 Hz, CH_3-18), 1.50 (1H, m, H-2a), 1.53 (3H, s, CH_3-15), 1.75 (1H, dd, *J* = 4.8, 15.0 Hz, H-9a), 1.82 (3H, s, CH_3-14), 2.14 (1H, dd, *J* = 11.4; 15.0 Hz, H-9b), 2.41 (1H, hept, *J* = 6.6 Hz, H-17), 2.61 (1H, d, *J* = 6.0, 10.2 Hz, H-2b), 3.39 (3H, s, OMe), 4.03 (1H, dd, *J* = 6.0, 10.2 Hz, H-1), 4.09 (1H, m, H-7), 5.39 (1H, m, H-6), 5.58 (1H, d, *J* = 2.4 Hz, H-13a), 5.59 (1H, m, H-8), 5.61 (1H, d, *J* = 1.8 Hz, H-5), 6.24 (1H, d, *J* = 3.0 Hz, H-13b). ^{13}C-NMR ($CDCl_3$, 150 MHz), δ_C: 18.7 (C-18), 19.1 (C-19), 22.5 (C-15), 27.3 (C-14), 34.1 (C-17), 35.2 (C-9), 41.6 (C-2), 49.7 (C-7), 58.7 (OMe), 70.5 (C-6), 75.5 (C-8), 81.9 (C-10), 86.4 (C-1), 103.6 (C-3), 122.7 (C-13), 128. 8 (C-5), 136.3 (C-12), 140.4 (C-4), 169.8 (C-11), 176.1 (C-16).

Tagitinin C (**5**): pale oil, $[\alpha]_D^{25}$ -163.2 (*c* 0.15, MeOH); (reference value: $[\alpha]_D^{28} -$ 187.5 (*c* 1.2, MeOH) [21]; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ_H: 1.06 (3H, d, *J* = 7.2 Hz, CH_3-19), 1.07 (3H, d, *J* = 7.2 Hz, CH_3-18), 1.54 (3H, s, CH_3-15), 1.96 (3H, d, *J* = 1.8 Hz, CH_3-14), 2.00 (1H, dd, *J* = 10.2, 14.4 Hz, H-9a), 2.42 (1H, m, H-9b), 2.44 (1H, hept, *J* = 7.2 Hz, H-17), 3.53 (1H, m, H-7), 5.36 (1H, m, H-8), 5.39 (1H, br d, *J* = 9.0 Hz, H-6), 5.80 (1H, d, *J* = 1.8 Hz, H-13a), 5.87 (1H, d, *J* = 9.0 Hz, H-5), 6.23 (1H, d, *J* = 17.4 Hz, H-2), 6.35 (1H, d, *J* = 1.8 Hz, H-13b), 6.94 (1H, d, *J* = 17.4 Hz, H-1). ^{13}C-NMR ($CDCl_3$, 150 MHz), δ_C: 18.6 (C-19), 18.8 (C-18), 19.7 (C-14), 29.2 (C-15), 34.1 (C-17), 47.1 (C-7), 48.5 (C-9), 72.2 (C-10), 73.8 (C-8), 75.9 (C-6), 124.5 (C-13), 129.7 (C-2), 136.1 (C-12), 137.2 (C-5), 139.0 (C-4), 160.4 (C-1), 169.6 (C-11), 176.2 (C-16), 196.6 (C-3).

***1β, 2α*-Epoxytagitinin C** (**6**): Colorless crystals, mp 111 °C, $[\alpha]_D^{25}$ -56.8 (*c* 0.03, $CHCl_3$); (reference value: $[\alpha]_D^{24} - 69$ (*c* 1.3, $CHCl_3$) [20]); ESI MS *m/z* 349.2 $[M+H]^+$, ^{1}H-NMR ($CDCl_3$, 600 MHz) δ_H: 1.11 (3H, d, *J* = 7.2 Hz, CH_3-19), 1.12 (3H, d, *J* = 7.2 Hz, CH_3-18), 1.28 (3H, s, CH_3-15), 1.95 (3H, t, *J* = 1.8 Hz, CH_3-14), 1.97 (1H, dd, *J* = 10.2, 15.0 Hz, H-9a), 2.12 (1H, dd, *J* = 4.2, 15.0 Hz, H-9b), 2.49 (1H, hept, *J* = 7.2 Hz, H-17), 3.22 (1H, d, *J* = 2.4 Hz H-1), 3.28 (1H, dd, *J* = 1.8, 4.2 Hz, H-7), 3.68 (1H, d, *J* = 2.4 Hz, H-2), 5.39 (1H, ddd, *J* = 3.0, 4.2, 10.2 Hz, H-8), 5.62 (1H, br d, *J* = 1.8 Hz, H-6), 5.84 (1H, d, *J* = 1.8 Hz, H-13a), 6.35 (1H, d, *J* = 1.8 Hz, H-13b), 6.37 (1H, d, *J* = 1.8 Hz, H-5). ^{13}C-NMR ($CDCl_3$, 150 MHz), δ_C: 18.7 (C-18; 19), 20.2 (C-14), 26.0 (C-15), 34.1 (C-17), 42.3 (C-9), 49.7 (C-7), 58.1 (C-2), 65.5 (C-1), 70.1 (C-10), 73.1 (C-8), 75.1 (C-6), 124.9 (C-13), 135.6 (C-12), 137.1 (C-4), 141.5 (C-5), 168.9 (C-11), 176.2 (C-16), 193.6 (C-3).

Tagitinin G (**7**): colorless crystals, $[\alpha]_D^{25}$ -112.8 (*c* 0.05, $CHCl_3$); (reference value: $[\alpha]_D^{30}$ -134 (*c* 0.30, $CHCl_3$) [22]). ^{1}H-NMR ($CDCl_3$, 600 MHz), δ_H: 0.96 (3H, d, *J* = 7.2 Hz, CH_3-15), 1.23 (3H, d, *J* = 6.6 Hz, H-13), 1.41 (3H, s, CH_3-14), 1.60 (1H, m, H-9a), 1.62 (1H, m, H-5a), 1.63 (1H, dd, *J* = 8.4, 12.6 Hz, H-2a), 1.98 (1H, m, H-4), 2.22 (1H, dd, *J* = 8.4, 12.6 Hz, H-2b), 2.25 (1H, ddd, *J* = 3.0, 5.4, 8.4 Hz,, H-5b), 2.37 (1H, dq, *J* = 6.6, 18.0 Hz, H-12), 2.50 (1H, ddd, *J* = 7.8, 10.2, 18.0 Hz, H-7), 2.92 (1H, dd, *J* = 8.4, 13.8 Hz, H-9b), 3.81 (1H, t, *J* = 7.8 Hz, H-8), 4.00 (1H, br s, H-1), 4.41 (1H, ddd, *J* = 1.2, 8.4, 10.2 Hz, H-6). ^{13}C-NMR ($CDCl_3$, 150 MHz) δ_C: 13.6 (C-13), 15.6 (C-15), 26.8 (C-14), 35.3 (C-4), 38.4 (C-5), 38.7 (C-9), 43.0 (C-12), 44.3 (C-2), 60.3 (C-7), 69.8 (C-8), 78.4 (C-1), 79.4 (C-6), 82.8 (C-10), 110.5 (C-3), 178.4 (C-11).

Tagitinin H (**8**): colorless crystals, $[\alpha]_D^{25}$ -53.6 (*c* 0.04, $CHCl_3$), (reference value: $[\alpha]_D^{22}$ -70 (*c* 0.20, $CHCl_3$) [22]); ^{1}H-NMR ($CDCl_3$, 600 MHz), δ_H: 0.99 (3H, d, *J* = 7.2 Hz, CH_3-15), 1.20 (3H, d, *J* = 6.6 Hz, H-13), 1.41 (3H, s, CH_3-14), 1.68 (1H, m, H-5a), 1.70 (1H, m, H-2a), 1.75 (1H, m, H-2b), 1.77 (1H, m, H-1a), 1.78 (1H, m, H-9a), 1.97 (1H, m, H-1b), 1.98 (1H, m, H-4), 2.22 (1H, m, H-5b), 2.24 (1H, m, H-9b), 2.34 (1H, dq, *J* = 6.6, 19.2 Hz, H-12), 2.51 (1H, ddd, *J* = 7.2, 9.6, 18.0 Hz, H-7), 3.77 (1H, t, *J* = 8.4 Hz, H-8), 4.44 (1H, dt, *J* = 9.6, 10.2 Hz, H-6). ^{13}C-NMR ($CDCl_3$, 150 MHz) δ_C: 13.6 (C-13), 15.6 (C-15), 28.5 (C-14), 33.3 (C-2), 35.6 (C-4), 37.9 (C-5), 38.6 (C-1), 43.0 (C-12), 47.2 (C-9), 60.1 (C-7), 69.5 (C-8), 78.5 (C-10), 80.0 (C-6), 110.3 (C-3), 178.6 (C-11).

2.4. Cytotoxic activity assay

The cytotoxic activities of compounds **1–8** were evaluated *in vitro* against KB, MCF-7, HepG2, and A549 cell lines. The cytotoxic activity was measured using a modified MTT assay, which was similar to those described in the previous papers [23].

3. RESULTS AND DISCUSSION

Compound **1** was obtained as white crystals, mp. 276–277 ºC and was optically active with an optical rotation value of $[\alpha]_D^{25}$ -56.1 (*c,* 0.1, MeOH). The ^{1}H-NMR spectrum of **1** showed the signals of three aromatic protons at δ_H 6.38 (1H, s), 7.00 (1H, s) and 6.83 (1H, d, *J* = 7.8 Hz), one methylenedioxy group at δ_H 6.03 (1H, br s), 6.01 (1H, d, *J* = 0.6 Hz), one oxygenated methylene group at δ_H 3.97 (1H, t, *J* = 9.0 Hz) and 4.63 (1H, t, *J* = 9.0 Hz), one *β*-glucopyranosyl moiety at δ_H 4.65 (d, *J* = 7.8 Hz, H-1″), 3.33 (m, H-2″), 3.28 (m, H-3″), 3.17 (m, H-4″), 3.16 (m, H-5″), and 3.41, 3.60 (m, CH_2- 6″) and the remaining protons in the aliphatic region. Analyses of the ^{13}C-NMR and DEPT spectra revealed the resonances of twenty-five carbon signals, including four methylene groups (one methylenedioxy, two sp^3 protonated carbons (one carbonyl at δ_C 167.9 and eleven sp^2 non-protonated carbons). The chemical shifts of C-4, C-5, C-6′, C-2′, C-3′, and C-4′ were characteristic of the oxygenated aromatic carbons (Table 1). The COSY spectrum of **1** indicated the presence of two spin-spin coupling systems as follows: H-7 (δ_H 2.70; 2.94)/ H-8 (δ_H 3.32)/ H-9 (δ_H 3.97, 4.63), and H-1″ (δ_H 4.65)/ H-2″ (δ_H 3.33)/ H-3″ (δ_H 3.28)/ H-4″ (δ_H 3.17)/ H-5″ (δ_H 3.16)/ H-6″ (δ_H 3.41, 3.60).

Table 1. ^{13}C NMR spectral data of compounds **1**-**2**.

Cnº	Compound 1		Compound 2	
	δ_C	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)
1	131.4	-	128.2	-
2	129.5	-	133.0	-
3	108.3	6.38 (1H, s)	109.9	6.51 (1H, s)
4	146.1	-	145.9	-
5	148.1	-	147.7	-
6	109.7	7.00 (1H, s)	110.2	7.04 (1H, s)
7	32.1	2.70 (1H, t, 15.6) 2.94 (1H, dd, 6.6, 15.0)	70.4	5.01 (1H, d, 3.6)
8	35.1	3.32 (1H, m)	37.2	2.87 (1H, m)
9	70.4	3.97 (1H, t, 9.0) 4.63 (1H, t, 9.0)	67.8	4.36 (1H, dd, 8.4; 10.2) 4.33 (1H, t, 8.4)

Cn°	Compound 1		Compound 2	
	δ_C	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)
10	101.5	6.03 (1H, br s) 6.01 (1H, br s)	101.3	6.02 (1H, d, 1.2) 6.01 (1H, d, 1.2)
1′	125.2	-	134.3	-
2′	148.1	-	110.7	6.53 (1H, d, 1.8)
3′	144.5	-	146.1	-
4′	145.7	-	146.6	-
5′	115.2	6.83 (1H, d, 7.8)	107.4	6.75 (1H, d, 7.8)
6′	147.2	-	123.5	6.36 (1H, dd, 1.8; 7.8)
7′	146.1	-	42.5	4.51 (1H, d, 5.4)
8′	120.1	-	39.9	3.40 (1H, dd, 5.4; 11.4)
9′	167.9	-	174.8	-
10′	-	-	100.8	5.95 (1H, d, 1.2) 5.94 (1H, d, 1.2)
1″	102.9	4.65 (1H, d, 7.8)	99.8	4.24 (1H, d, 7.8)
2″	73.3	3.33 (1H, m)	73.6	3.02 (1H, m)
3″	76.0	3.28 (1H, m)	76.5	3.11 (1H, m)
4″	69.7	3.17 (1H, m)	70.4	3.04 (1H, m)
5″	77.2	3.16 (1H, m)	77.0	3.12 (1H, m)
6″	60.5	3.41 m 3.60 (1H, m)	61.3	3.75 (1H, dd, 4.2; 11.4) 3.46 (1H, m)
2″-OH	-	4.50 (1H, br s)	-	4.99 (1H, d, 4.8)
3″-OH	-	5,10 (1H, br s)	-	4.93 (1H, br s)
4″-OH	-	4.99 (1H, br s)	-	4.92 (1H, br s)
6″-OH	-	4.50 (1H, br s)	-	4.66 (1H, t, 5.4)
Ar-OH	-	7.13 (1H, br s)	-	-
Ar-OH	-	6.69 (1H, br s)	-	-

In the HMBC spectrum of **1**, the position of the methylenedioxy group at C-4/C-5 was determined by the HMBC cross-peaks of the proton CH_2-10 (δ_H 6.01 and 6.03) with C-4 (δ_C 146.1) and C-5 (δ_C 148.1). The HMBC correlations of the protons CH_2-9 with carbonyl carbon (δ_C 167.9 (C-9′), C-8′ (δ_C 120.1), and C-8 (δ_C 35.1) indicated the presence of a lactone ring. Furthermore, the HMBC correlations from the anomeric proton (δ_H 4.65) with C-3′ (δ_C 144.5) confirmed that the glucopyranosyl moiety was placed at C-3′. Thus, based on the NMR spectra analyses and comparison with the reported data, compound **1** was identified as cleiseberharside A [17]. This lignan compound was first isolated from the *Tithonia* genus.

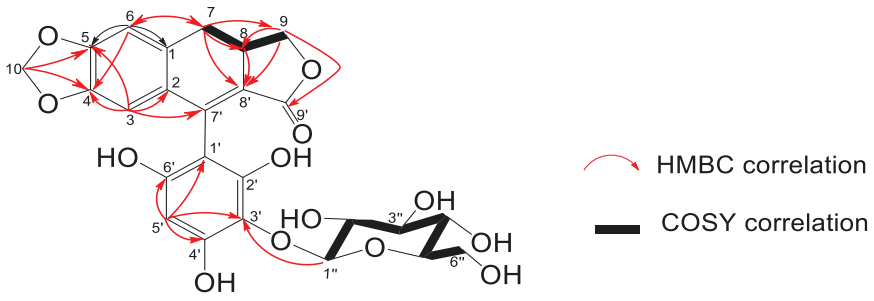

Figure 2. Key ^{1}H-^{1}H COSY and HMBC correlations of **1**.

Compound **2** was obtained as a white solid and was optically active with an optical rotation value of $[\alpha]_D^{25}$ -65.4 (*c* 0.1; MeOH). The ^{1}H-NMR spectrum of **2** presented the signals of two singlet aromatic protons at δ_H 6.51 (H-3) and 7.04 (H-6), an ABX ring system at δ_H 6.53 (d, *J* = 1.8 Hz, H-2′), 6.75 (d, *J* = 7.8 Hz, H-5′), and 6.36 (dd, *J* = 1.8, 7.8 Hz, H-6′), two methylenedioxy groups at δ_H 6.02 (1H, d, *J* = 1.2 Hz, H-10a), 6.01 (1H, d, *J* = 1.2 Hz, H-10b) and 5.95 (1H, d, *J* = 1.2 Hz, H-10′a), 5.94 (1H, d, *J* = 1.2 Hz, H-10′b), one oxygenated methylene group at δ_H 4.36 (1H, dd, *J* = 8.4, 10.2 Hz, H9a), 4.33 (1H, t, *J* = 8.4 Hz, H-9b), four methine groups at δ_H 5.01 (1H, d, *J* = 3.6, H-7), 2.87 (1H, m, H-8), 4.51 (1H, d, *J* = 5.4 Hz, H-7′), 3.40 (1H, dd, *J* = 5.4, 11.4 Hz, H-8′), and one *β*-glucopyranosyl moiety at δ_H [4.24 (1H, d, *J* = 7.8 Hz, H-1″), 3.02 (1H, m, H-2″), 3.11 (1H, m, H-3″), 3.04 (1H, m, H-4″), 3.12 (1H, m, H-5″), 3.75 (1H, dd, *J* = 4.2, 11.4 Hz, H-6″a), 3.46 (1H, m, H-6″b)]. The ^{13}C-NMR and DEPT spectra of **2** exhibited the presence of twenty-six carbons, including one carbonyl group (δ_C 175.8 (C-9′)), two methylenedioxy groups at δ_C 101.3 (C-10), 100.8 (C-10′), two methylene groups, fourteen methine groups (five sp^2 methines and nine sp^3 methines) and seven non-protonated aromatic carbons. The chemical shifts of C-4, C-5, C-7, C-9, C-3′, and C-4′ suggested their linkages to oxygen atoms. The proton H-8′ exhibited a strong coupling constant (*J* = 11.4 Hz) and a smaller one (*J* = 5.4 Hz), while H-7′ appeared as a doublet (*J* = 5.4 Hz), indicating a *trans*-pseudodiaxial relationship between H-8′ and H-8, as well as a pseudoequatorial position for H-7′. Furthermore, H-7 displayed a gauche coupling constant (*J* = 3.6 Hz), suggesting a pseudoequatorial orientation. Thus, based on detailed analyses of the 1D-NMR spectra of **2** and comparison with the reported data, compound **2** was identified as cleisindoside A [17, 18]. Compound **2** is a lignan compound, also isolated for the first time from the *Tithonia* genus.

Compound **3** was obtained as colorless crystals. The ^{1}H-NMR spectrum of **3** showed the signals of four methyl groups [one singlet at δ_H 1.45 (3H, s, H-15), three doublets at δ_H 1.12 (3H, d, *J* = 7.2 Hz, H-14), 1.07 (3H, d, *J* = 6.6 Hz, H-19), 1.04 (3H, d, *J* = 6.6 Hz, H-18)], one exo-methylene group at δ_H 5.52 (1H, d, *J* = 3.6 Hz, H-13a), 6.26 (1H, d, *J* = 3.6 Hz, H-13b), two oxymethine groups at δ_H 4.56 (1H, ddd, *J* = 1.8, 6.6, 9.6 Hz, H-6), 5.54 (1H, m, H-8) and the remaining protons in the aliphatic region at δ_H 1.76- 4.08. Analyses of the ^{13}C-NMR and DEPT spectra of compound **3** revealed the presence of nineteen carbons, including two carbonyl groups at δ_C 176.2 (C-16), 169.4 (C-11), four methyl groups, four sp^3 methylene groups, one sp^2 methylene group, five sp^3 methine groups (two oxygenated methine at δ_C 81.2 (C-6) and 69.6 (C-8)), and three non-protonated carbons at δ_C 108.8 (C-3), 80.2 (C-10) and 137.0 (C-12). Thus, analyses of the NMR spectra and comparison with the reported data led to the identification of compound **3** as tirotundin. This compound was identified as a potential neurotoxin to nematodes with an IC_{50} value of 6.89 ± 0.30 µg/mL [17].

Compound **4** was obtained as a colorless oil. The ^{1}H-NMR spectrum of **4** indicated similar signals to those of **3**, except that the addition of one methoxy group at δ_H 3.39 (s, OCH_3), one oxygenated methine group at δ_H 4.03 (1H, dd, J = 6.0, 10.2 Hz, H-1), one sp^2 methine group at δ_H 5.61 (d, J = 1.8 Hz, H-5) and the disappearance of several signals in the aliphatic region at H-4, H-5 that were observed in the structure of **4**. The ^{13}C-NMR and DEPT spectra of **4** exhibited the characteristic signals of a sesquiterpene lactone compound with the presence of twenty carbons, including four methyl groups at δ_C 18.7 (C-18), 19.1 (C-19), 22.5 (C-15), 27.3 (C-14), one methoxy group at δ_C 58.7 (OMe), three methylene groups at δ_C 41.6 (C-2), 35.2 (C-9), 122.7 (C-13), six methine groups at δ_C 86.4 (C-1), 128.8 (C-5), 70.5 (C-6), 49.7 (C-7), 75.5 (C-8), 34.1 (C-17), four non-protonated carbons at δ_C 103.6 (C-3), 140.4 (C-4), 81.9 (C-10), 136.3 (C-12), and two carbonyl groups at δ_C 176.1 (C-16), 169.8 (C-11). Analyses of the 1D-NMR spectral data of compound **4**, and comparison with the reference data determined the structure of **4** as 2β-methoxydesoxytagitinin B [20].

Compound **5** was isolated as a pale oil. The ^{1}H-NMR spectrum of **5** indicated the signals of a sesquiterpene lactone compound, including three olefinic protons at δ_H 5.87 (1H, d, J = 9.0 Hz, H-5), 6.23 (1H, d, J = 17.4 Hz, H-2), 6.94 (1H, d, J = 17.4 Hz, H-1), one exomethylene group at 5.80 (1H, d, J = 1.8 Hz, H-13a), 6.35 (1H, d, J = 1.8 Hz, H-13b), four methyl groups at δ_H 1.06 (3H, d, J = 7.2 Hz, CH_3-19), 1.07 (3H, d, J = 7.2 Hz, CH_3-18), 1.54 (3H, s, CH_3-15), 1.96 (3H, d, J = 1.8 Hz, CH_3-14), and the aliphatic protons in the region at δ_H 2.00- 5.36 ppm were also observed. The ^{13}C-NMR and DEPT spectra of **5** revealed the presence of nineteen carbons, including four methyl groups at δ_C 18.6 (C-19), 18.8 (C-18), 19.7 (C-14), 29.2 (C-15), three carbonyl groups at δ_C 169.6 (C-11), 176.2 (C-16), 196.6 (C-3), two methylene groups at δ_C 48.5 (C-9), 124.5 (C-13), seven methine groups [three sp^2 methine at δ_C 129.7 (C-2), 137.2 (C-5), 160.4 (C-1)], and three non-protonated carbons at δ_C 72.2 (C-10), 136.1 (C-12), 139.0 (C-4). Comparison of the 1D-NMR data with reference data determined the structure of **5** as tagitinin C. Tagitinin C exhibits anticancer activity and has been shown to inhibit the proliferation of human glioblastoma U373 cells with an IC_{50} of 6.1 µg/mL [24] and HL-60 cells with an IC_{50} of 1.1 µM [25].

Compound **6** was isolated as colorless crystals. Its ESI-MS showed the pseudo-molecular ion $[M+H]^+$ at m/z 349.2. Comparison of the 1D-NMR spectra of **6** with those of **5** indicated the structural similarities between **5** and **6**. Compound **6** possessed the same sesquiterpene lactone skeleton as compound **5**, except that the disappearance of the *trans*-double bond between C-1 and C-2 in the structure of **5** was replaced by two oxymethine groups at δ_C 65.5 (C-1), 58.1 (C-2) and at δ_H 3.22 (1H, d, J = 2.4 Hz, H-1), 3.68 (1H, d, J = 2.4 Hz, H-2) in the structure of **6**. Detailed analyses of the 1D-NMR spectral data of compound **6** and comparison with the reference data established the structure of **6** as 1β,2α-epoxytagitinin C. Compound *1β,2α*-epoxytagitinin C **(6)** was previously reported as a potential anti-leukemia agent [26].

Compound **7** was isolated as colorless crystals. In the ^{1}H NMR spectrum of **7**, the signals of three methyl groups at δ_H 1.23 (3H, d, J = 6.6 Hz, CH_3-13), 1.41 (3H, s, CH_3-14), and 0.96 (3H, d, J = 7.2 Hz, CH_3-15), two oxygenated methines at δ_H 3.81 (1H, t, J = 7.8 Hz, H-8), 4.41 (1H, ddd J = 1.2, 8.4, 10.2 Hz, H-6) and the remaining signals in the aliphatic region at δ_H 1.62- 4.41 were observed. The ^{13}C-NMR and DEPT spectra of **7** showed the presence of fifteen carbons, including one carbonyl group at δ_C 178.4), three methyl groups at δ_C 13.6, 15.6 and 26.8), three methylene groups at δ_C 44.3 (C-2), 38.4 (C-5), 38.7 (C-9), six methine groups (three oxymethines at δ_C 78.4 (C-1), 79.4 (C-6), 69.8 (C-8)) and two oxygen-bearing carbons at δ_C 82.8 (C-10), 110.5 (C-3). In the ^{1}H-^{1}H COSY spectrum of **7**, three spin-spin interaction systems of protons: H-1 (δ_H 4.00)/H-2 (δ_H 2.22, 1.63), H-14 (δ_H 0.96)/H-4 (δ_H 1.98)/H-5 (δ_H 2.25, 1.62)/H-6 (δ_H 4.41)/H-7 (δ_H

2.50)/H-8 (δ_H 3.81)/H-9 (δ_H 2.92, 1.60)/ and H-7 (δ_H 2.50)/H-12 (δ_H 2.37)/H-13 (δ_H 1.23) were noted. In the HMBC spectrum of **7**, the HMBC correlations between the proton of CH_3-13 (δ_H 1.23) with C-12 (δ_C 43.0), the proton of CH_3-14 (δ_H 1.41) with C-4 (δ_C 35.3), the protons of CH_3-15 (δ_H 0.96) with C-10 (δ_C 82.8), determined the positions of the methyl groups at C-12, C-4, C-10, respectively. Additionally, the C-O-C linkage between C-3 and C-8 was confirmed by the HMBC correlations from H-8 (δ_H 3.81) to C-3 (δ_C 110.5). Based on the above evidence and comparison of the 1D-NMR data of **7** with the reference data, its structure was determined as tagitinin G [22].

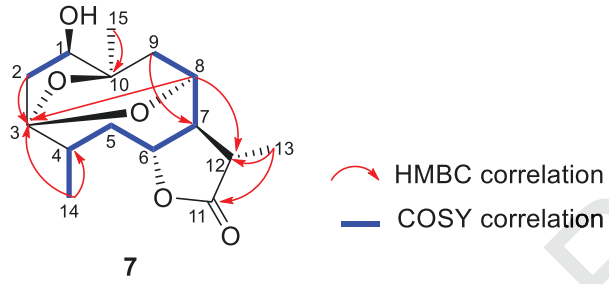

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Figure 3. Key ^{1}H-^{1}H-COSY and HMBC correlations of **7**.

Compound **8** was isolated as colorless crystals. The 1D-NMR spectra of **8** indicated similar signals with those of **7**, except that the absence of one oxymethine group in **8** was replaced by one methylene group at δ_H 1.97 (1H, m, H-1b), 1.77 (1H, m, H-1a) and at δ_C 38.6 (C-1). Detailed analyses of the NMR spectra of **8** and comparison with the literature data determined the structure of **8** as tagitinin H [20].

All the isolated compounds (**1**-**8**) were evaluated for their cytotoxic activity against four human cancer cell lines (human mouth epidermal carcinoma (KB), human breast carcinoma (MCF7), human hepatocellular carcinoma (Hep-G2), and human lung carcinoma (A549)) using a modified MTT assay [23]. Among the tested compounds, compounds **4**-**6** demonstrated strong activity against all four cell lines, with IC_{50} values ranging from 0.28 to 3.76 µM. Notably, compound **5** exhibited the strongest cytotoxicity against four tested human cancer cell lines, KB, MCF7, HepG2, A549, with the IC_{50} values of 0.28, 1.18, 1.38, 1.15 µM, respectively. These values were comparable to those of the reference compound, ellipticine (Table 2). Conversely, four compounds **2**, **3**, **7** and **8** which possessed similar sesquiterpene lactone structures, showed no significant activity against the four human cancer cell lines. A comparison of cytotoxicity among the isolated sesquiterpene lactones suggested that the presence of the α-methylene-γ-lactone or especially the olefinic bonds at C-1/C-2 and C-4/C-5, forming an α,β-unsaturated carbonyl (both motifs are well-known Michael acceptors) in compounds **4**, **5**, **6** seemed to enhance their biological activities [4]. In contrast, inactive compounds (compound **3**, **7** and **8**) lacked one or both of these features, indicating that these structural motifs are key contribution to the observed cytotoxicity. In addition, compound **1** exhibited moderate activity against MCF7 and HepG-2 cell lines with the IC_{50} values of 17.77 and 13.36 µM, respectively. Compound **2** was inactive against all four tested human cancer cell lines.

To the best of our knowledge, this is the first time of the isolation of two lignan compounds, **1** and **2**, from the *Tithonia* genus. Furthermore, our study highlights the potential of *Tithonia diversifolia* as a source for the development of novel therapeutic agents. The significant cytotoxic effects observed, particularly with tagitinin C, strongly supported its potential as an anticancer agent.

Table 2. *In vitro* cytotoxic activity of compounds **1-8** (IC_{50} values were expressed in μM).

Compounds	KB	MCF7	HepG2	A549
1	> 128	17.77 ± 3.24	13.36 ± 4.0	> 128
2	> 128	> 128	> 128	> 128
3	> 128	> 128	> 128	> 128
4	3.13 ± 0.16	3.63 ± 0.13	3.71 ± 0.16	3.76 ± 0.16
5	0.28 ± 0.02	1.18 ± 0.08	1.38 ± 0.29	1.15 ± 0.06
6	1.95 ± 0.03	2.91 ± 0.11	3.51 ± 0.08	3.27 ± 0.08
7	> 128	> 128	> 128	> 128
8	> 128	> 128	> 128	> 128
Ellipticine	1.75 ± 0.08	1.79 ± 0.08	1.79 ± 0.08	1.75 ± 0.08

4. CONCLUSIONS

From the ethyl acetate extract of the *Tithonia diversifolia* flowers, eight known compounds were isolated and structurally characterized, including two lignans cleiseberharside A (**1**), cleisindoside A (**2**), and six sesquiterpene lactones tirotundin (**3**), 2*β*-methoxydesoxytagitinin B (**4**), tagitinin C (**5**), 1*β*,2*α*-epoxytagitinin C (**6**), tagitinin G (**7**), and tagitinin H (**8**). Their structures were elucidated by spectroscopic analyses, including 1D-, 2D-NMR spectra, and by comparison with the previously reported data. Among the tested compounds, compounds **4**-**6** showed strong cytotoxicity against KB, MCF-7, HepG-2, and A549 human cancer cell lines, with IC_{50} values ranging from 0.28 - 3.76 μM. Compound **5**, in particular, exhibited the strongest cytotoxicity against all four tested human cancer cell lines (KB, MCF7, HepG2, A549) with the IC_{50} values of 0.28, 1.18, 1.38, 1.15 μM, respectively, comparable to the reference compound ellipticine. Along with the discovery of lignans **1** and **2** for the first time from the *Tithonia* genus, these findings provide valuable insights into the bioactive constituents of *Tithonia diversifolia*, offering a solid foundation for future research into their mechanisms of action and potential clinical applications.

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Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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