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ORIGINAL ARTICLE

Two new cycloartanes from the leaves of *Combretum quadrangulare* growing in Vietnam and their biological activities

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Abstract Two new cycloartanes, combretanones G and H (**1** and **2**), were isolated from the leaves of *Combretum quadrangulare*. Their structures were elucidated by applying a set of spectroscopic methods, while their relative configurations were determined using DFT-NMR chemical shift calculations and subsequent assignment of DP4 probabilities. Compounds **1** and **2** are C-23/C-24 stereoisomers of the previously-reported euphonerin E. Both exhibited moderate cytotoxicity

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Cytotoxicity;
Antiparasitic activity

against three human cancer cell lines. Compound **2** was shown to be a potent antiparasitic. Our results confirm the traditional medicinal uses of *Combretum quadrangulare* in Vietnam.

1. Introduction

Combretum quadrangulare Kurz (Combretaceae) is a perennial tree that is widespread throughout Eastern Asia. Known as "Tram Bau" in Vietnam, this species is widely used in folk medicine and is claimed to have ethnopharmacological properties as a hepatoprotective, antipyretic, analgesic, antidysenteric, and anthelmintic. In Vietnam, the seeds of *Combretum quadrangulare* have traditionally been used to suppress *Toxocara canis* larvae. The wide ethnopharmacological use of *C. quadrangulare* paved the way for phytochemical investigations that reported the presence of numerous triterpenes (mostly cycloartanes, ursanes, lupanes, and oleananes), along with a limited number of flavonoids (Adnyana et al., 2000, 2001; Banskota et al., 1998, 2000a, 2000b; Ganzera et al., 1998; Toume et al., 2011). As part of our ongoing investigation of the biochemical properties of Vietnamese medicinal plants (Duong et al., 2017, 2018a, 2019; Pham et al., 2020), in this study the phytochemical properties of EtOH extracted from the leaves of *C. quadrangulare* were investigated using bioactive-guided isolation. We report the isolation and structural elucidation of two new cycloartanes: combretanones G and H (**1**–**2**). The relative configurations of **1** and **2** were determined using GIAO NMR chemical shift calculations followed by calculation of DP4 probability. Compounds **1** and **2** were evaluated for cytotoxicity against three human cancer cell lines and human Adipose-derived cell line (hAdCs). Compound **2** was assayed for anti-parasitic activity against *Toxocara canis* larvae.

2. Material and methods

2.1. General experimental procedures

The NMR spectra were recorded on a Bruker Avance III spectrometer (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) using residual solvent signals as internal references. The HR–ESI–MS was recorded on an HR–ESI–MS MicrOTOF–Q mass spectrometer with an LC-Agilent 1100 LC-MSD Trap spectrometer. Thin layer chromatography (TLC) was carried out on precoated silica gel 60 F_{254} or silica gel 60 RP–18 F_{254S} (Merck), and spots were visualized by spraying with 10% H_2SO_4 solution followed by heating. Gravity column chromatography was performed on silica gel 60 (0.040–0.063 mm, Himedia).

2.2. Plant material

Leaves of *C. quadrangulare* were collected in Duc Hoa, Long An Province in March-April 2016. The plant was identified as *C. quandrangulare* Kurz by Dr. Cong Luan Tran, Tay Do University, Can Tho, Vietnam. A voucher specimen (No UE-002) was deposited in the herbarium of the Department of Organic Chemistry, Faculty of Chemistry, Ho Chi Minh University of Education, Ho Chi Minh City, Vietnam.

2.3. Extraction and isolation of compounds

Dried leaves of *C. quadrangulare* (3.5 kg) were crushed and extracted with 10 L of EtOH (three times) at 70 °C for 8 h. The filtrated solution was evaporated to dryness under reduced pressure to obtain a crude extract (118.4 g). This crude extract was successively partitioned by *n*-hexane, *n*-hexane: EtOAc (1:1), EtOAc, and *n*-butanol to produce fractions H (6.1 g), HEA (54.5 g), EA (30.0 g), and BU (12.0 g), respectively. Fraction HEA (54.5 g) was subjected to silica gel column chromatography, using an isocratic mobile phase consisting of *n*-hexane: EtOAc: acetone (5:1:1) to obtain fractions P1 (2.95 g), P2 (0.72 g), P3 (0.94 g), P4 (0.82 g), P5 (0.69 g), P6 (0.23 g), P7 (0.2 g), P8 (0.15 g), P9 (0.3 g), P10 (0.1 g), P11 (3.0 g), P12 (6.0 g), P13 (13.1 g), and P14 (20.1 g). Fraction P12 (6.0 g) was subjected to silica gel column chromatography, using an isocratic mobile phase consisting of a *n*-hexane: EtOAc: acetone solvent system 5/1/1, v/v/v), affording subfractions T1 (1.3 g), T2 (200.0 mg), T3 (300.0 mg), T4 (1.0 g), T5 (1.2 g), T6 (305.0 mg), T7 (120.0 mg), and T8 (0.5 g). Subfraction T6 (305.0 mg) was subjected to CC using the solvent system *n*-hexane: $CHCl_3$: EtOAc: acetone: H_2O (3:1:2:2:0.01) to give subfractions T6.1 (130.0 mg), T6.2 (60.0 mg), and T6.3 (30.0 mg). Subfraction T61 was rechromatographed and eluted with the same solvent system to yield **1** (1.4 mg) and **2** (4.7 mg).

2.3.1. Combretanone G (*1*)

White amorphous powder; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 1; HRESIMS *m/z*: $[M + Na]^+$ 495.3439 for $C_{30}H_{48}O_4Na$ (calcd. 495.3450).

2.3.2. Combretanone H (*2*)

White amorphous powder; ^{1}H NMR ($CDCl_3$ and acetone d_6, 500 MHz) and ^{13}C NMR ($CDCl_3$ and acetone d_6, 125 MHz) see Table 1; HRESIMS *m/z*: $[M + H]^+$ 473.3615 for $C_{30}H_{49}O_4$ (calcd. 473.3631).

2.4. Cytotoxicity assay

The cytotoxicity of **1** and **2** was evaluated against K562 (chronic myelogenous leukemia), HepG2 (liver hepatocellular carcinoma), MCF-7 (breast cancer), and hAdCs (human Adipose-derived) cell lines cultured in RPMI 1640 and DMEM media. The method followed that in Duong et al. (2019).

2.5. Antiparasitic activity assay

Toxocara canis larvae were prepared in our laboratory using the method reported in Nguyen et al. (2017). Adult worms were collected from pubs. For egg production, male and female worms were cultured together in PBS supplemented with 1% human serum plus penicillin (100 U/mL) and streptomycin (100 μg /mL) at 37 °C under a 5% CO_2 atmosphere for up to 7 days. The eggs were then collected by centrifugation and incubation in 1% formalin–PBS for 30 days at room

Table 1 ^{1}H NMR (500 MHz, δ_H, multi, (*J* in Hz) and ^{13}C NMR (125 MHz) spectral data of compounds **1** and **2**.

No.	**1** ($CDCl_3$)		Euphornerin E ($CDCl_3$)[27]		**2** ($CDCl_3$)		**2** (acetone d_6)	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	1.60 m, 1.85 m	32.7		32.8	1.62 m, 1.89 m	32.6	1.55 m, 1.73 m	32.4
2	2.32 ddd (2.5,4.5,14.0) 2.70 td (6.5, 13.5)	37.2		38.7	2.33 ddd (1,5, 3.5, 14.5) 2.70 td (6.5, 14.0)	37.4	2.2 ddd (2.5, 4.5, 13.0) 2.72 td (7.0, 14.0)	37.7
3		215.9		215.8		215.8		215.1
4		49.8		49.6		49.7		50.1
5	1.90 m	46.8		46.6	1.91 m	46.9	1.90 m	46.7
6	1.15 m, 1.73 m	31.3		31.1	1.15 m, 1.73 m	31.3	1.08 m, 1.68 m	29.2
7	3.65 ddd (10.5, 9.5, 4.0)	70.8	3.61 ddd (10.7, 8.9, 3.3)	70.6	3.64 m	70.8	3.73 ddd (3.0, 7.5, 12.5)	70.5
8	1.69 m	54.8		54.6	1.69 m	54.7	1.67 d (8.5)	55.3
9		21.0		20.8		21.0		21.0
10		26.7		26.5		26.5		27.3
11	1.32 m, 1.90 m	27.0		27.0	1.33 m, 1.90 m	26.6	1.28 m, 1.91 m	28.1
12	1.64 m, 1.85 m	33.0		32.5	1.63 m, 1.87 m	33.0	1.69 m	33.5
13		46.1		46.0		46.1		46.7
14		48.6		48.3		48.5		47.7
15	1.49 m, 1.60 m	37.4		37.0	1.49 m, 1.60 m	37.1	1.52 m, 1.58 m	37.6
16	1.37 m, 1.96 m	28.4		28.6	1.38 m, 1.96 m	28.9	1.39 m, 1.95 m	29.2
17	1.68 m	53.4		52.0	1.55 m	52.1	1.54 m	53.4
18	1.03 s	17.6	1.03 s	17.4	1.05 s	17.6	1.02 s	18.0
19	0.56 d (4.5) 0.95 d (4.5)	27.7	0.56 d (4.5) 0.95 d (4.5)	27.7	0.57 d (4.5) 0.95 m	27.7	0.62 d (4.0) 0.94 d (4.0)	27.7
20	1.72 m	33.5		32.5	1.75 m	32.3	1.65 m	33.0
21	0.90 d (6.5)	18.4	0.90 d (6.5)	18.1	0.92 d (6.5)	18.3	0.92 d (6.5)	19.0
22	1.15 m, 1.66 m	39.6		39.6	1.10 m, 1.56 m	37.6	0.98 m, 1.56 m	39.1
23	3.86 ddd (10.5, 9.0, 2.0)	76.4	3.70 ddd (11.0, 6.7, 2.0)	69.5	3.81 m	69.6	3.70 ddd (11.5, 7.0, 2.0)	70.1
24	3.96 d (8.5)	85.7	3.78 d (6.7)	80.2	4.09 dd (4.5, 3.5)	79.1	3.94 dd (4.5, 3.5)	80.0
25		141.9		144.6		144.5		147.0
26	4.94 brs, 5.02 s	114.3	4.93 brs, 4.99 s	114.2	4.98 brs, 5.05 brs	112.8	4.97 brs, 4.83 s	112.0
27	1.77 s	17.6	1.72 s	17.9	1.76 s	17.6	1.73 s	18.8
28	1.05 d (3.0)	22.3	1.03 d	22.6	1.05 d (2.0)	22.4	1.02 s	22.7
29	1.10 s	20.8	1.08 s	20.6	1.11 s	20.8	1.08 s	21.7
30	0.94 s	19.0	0.92 s	18.9	0.95 s	19.1	0.96 s	19.2
7-OH							3.14 d (5.5)[a]	
23-OH							3.19 d (6.0) [a]	
24-OH							3.79 d (4.0)	

[a] These signals were not assigned.

temperature in a sterile flask. Embryonic development was determined using an inverted microscope. For hatching, eggs were incubated using 6% sterile NaClO solution for 5 min at room temperature, then washed several times with sterile PBS buffer. Eggs were incubated with sterile Hank's balance saline solution pH 2.0 for 30 min at room temperature. The treated eggs were washed and incubated in serum-free DMEM medium supplemented with penicillin (100 U/mL) and streptomycin (100 µg/mL) at 37 °C in a 5% CO_2 atmosphere. The eggs hatched within three days. Larvae were concentrated by centrifugation and living larvae were selected by passing overnight through a 40 µm cell strainer in DMEM medium. HEA extract and compound **2** were tested for *in vitro* antiparasitic activity against *T. canis* larvae, using a method adapted from [3]. Briefly, twenty to thirty larvae were prepared in 200 µL of serum-free DMEM medium in each well of a 96 well–plastic cell culture plate. HEA extract and compound **2** were dissolved in DMSO at a concentration 10 mg/mL, diluted, then placed into wells at final concentrations of 250 µg/mL, 50 µg/mL, and 5 µg/mL. Albendazole and mebendazole prepared in DMSO were used as positive controls in the same concentrations, and DMSO was used as a solvent control. Larval movement was observed and scored (Table 2) on days 2 and 4 following exposure to the test substances. As shown in Table S1, the mobility index (MI) was calculated using Eq. (1), and the relative mobility (RM) using Eq. (2). The experiment was done in triplicate.

2.6. Computation

Conformational searching was performed using the xTB package. The energies of all conformers were calculated using the GFN2-xTB method. Stable conformers were identified using a quantum mechanical method at the b3lyp/6-31G(d,p) level of theory. GIAO calculations were performed at B3LYP/6-311 + G(d,p) (Grimblat et al., 2015). The DP4 probabilities were performed as reported in Duong et al. (2020a, 2020b).

Table 2 Antiparasitic activity of HEA extract and compound **2** on lavae of *T. canis in vitro* on day 2 and day 4.

Compound/extract	Relative mobility (%)					
	Day 2			Day 4		
	Concentration (μg/ml)			Concentration (μg/ml)		
	5	50	250	5	50	250
Albendazole	100.0	97.0	102.2	98.6	93.7	70.1
Mebendazole	99.6	100.0	100.4	99.3	100.0	100.0
HEA	98.1	99.3	81.3	88.9	92.3	77.5
2	72.4	44.5	39.9	50.2	34.9	35.1

[a]Data show average of three experiments.

3. Results and discussion

Compounds **1** and **2** were isolated from Fraction T6.1 (see the Experimental section) of the *C. quadrangulare* ethanol extract, based on bioactive-guided isolation (Tables 4–6). Compound **1** was determined to have the molecular formula $C_{30}H_{48}O_4$, based on HRESIMS data (*m*/*z* 495.3439, calcd. for $C_{30}H_{48}O_4Na$). This indicated seven degrees of unsaturation. The ^{1}H NMR spectrum showed six methyl groups (δ_H 1.77, 1.10, 1.05, 1.03, 0.94, and 0.90, the latest doublet with J = 6.5 Hz), three oxymethine protons [δ_H 3.96 (1H, d, J = 8.0 Hz), 3.86 (1H, ddd, J = 10.5, 9.0, 4 Hz), and 3.65 (1H, d, J = 8.0 Hz)], one sp^2 methylene [δ_H 5.02 (1H, br s) and 4.96 (1H, br s)], and two characteristically upfield-shifted doublets [δ_H 0.92 (1H, d, J = 4.5 Hz), and 0.57 (1H, d, J = 4.5 Hz)] assignable to cyclopropyl methylene protons. The ^{13}C NMR spectrum and HSQC spectrum revealed 30 carbons: carbonyl carbon (δ_C 215.0), one sp^2 quaternary carbon (δ_C 149.0), one sp^2 methylene (δ_C 114.3), three oxygenated sp^3 tertiary carbons (δ_C 85.7, 76.4, and 70.8), five sp^3 quaternary carbons (δ_C 50.0, 49.8, 48.6, 26.6, and 20.6), four sp^3 methine carbons (δ_C 54.6, 53.4, 46.7, and 33.3), eight sp^3 methylene carbons (δ_C 39.6, 37.6, 37.2, 32.4 (×2), 31.1, 28.4, and 26.8), and six methyl groups (δ_C 22.7, 22.1, 18.8, 18.1, and 17.4 (×2)). These spectroscopic features suggested the presence of five rings and were therefore diagnostic of a cycloartane-type triterpene (Khuong-Huu et al., 1975).

Detailed analysis of the COSY and HMBC results determined that compound **1** had a planar structure. A ketone moiety was suggested at C-3, based on HMBC correlations from the methylene protons H_2-1 (δ_H 1.85/1.60) and H_2-2 (δ_H 2.70/2.32), and the methyl protons H_3-28 (δ_H 1.10) and H_3-29 (δ_H 1.05) to C-3 (δ_C 215.0). The H-5/H-6, H-6/H-7, and H-7/H-8 COSY cross-peaks, along with long-range heteronuclear correlations from H-5, H-6, and H-8 to C-7 (δ_C 70.6) suggested the presence of a hydroxyl group at C-7. The presence of 23– and 24-OH substituents was established, based on the magnitude of the coupling constants between H-23 [δ_H 3.86 (1H, ddd, J = 10.5, 8.0, 2.0 Hz)] and H-24 [δ_H 3.96 (1H, d, J = 8.0 Hz)]. The HMBC correlations from the *exo*-olefinic protons at δ_H 5.02 and 4.94 to C-24 (δ_C 85.7), C-25 (δ_C 141.9), and C-27 (δ_C 17.6) ascribed this moiety to C-25. The relative configuration of **1** was assigned based on NOESY correlations and spin coupling analysis. The axial orientations of H-7 and H-8 were determined from their large coupling constants ($J_{H\text{-}7/H\text{-}8}$ 9.5 Hz) and this was confirmed by a NOESY correlation between H-7 and H_3-30. In particular, the key NOESY cross peaks of H-19β with both H_3-29 and H-8, and H-8 with H_3-18, determined their β-orientation, while the key NOESY correlations of H_3-28/H-5, H-5/H-7, H-7/H_3-30, and H_3-30/H-17 determined their

Table 3 IC_{50} and IC_{90} values (μg/mL) of the cytotoxicity of compounds **1** and **2**[a].

Tested compounds	K562		HepG2		MCF-7		hAdCs	
	IC_{50}	**IC_{90}**	**IC_{50}**	**IC_{90}**	**IC_{50}**	**IC_{90}**	**IC_{50}**	**IC_{90}**
1	13.3 ± 1.1	23.2 ± 0.2	20.0 ± 0.4	40.5 ± 1.8	65.8 ± 3.4	96.2 ± 0.6	38.9 ± 1.9	50.2 ± 3.4
2	21.0 ± 1.2	43.6 ± 0.7	37.3 ± 2.0	76.1 ± 2.9	70.3 ± 0.7	97.7 ± 0.2	79.2 ± 3.9	> 100
Doxorubicin	2.2 ± 0.7	35.2 ± 1.1	2.4 ± 0.2	26.2 ± 1.4	13.9 ± 2.2	75.3 ± 1.5	3.1 ± 0.8	46.9 ± 3.2

[a] Disclosed values are means ± standard errors of three independent experiments.

Table 4 Cytotoxicity (IC_{50}, IC_{90}) of extracts EA, H, HEA, and BU against HepG2.

Extract	EtOH	EA	H	HEA	BU
IC_{50} (μg/mL)	46.7 ± 2.8	> 100	39.4 ± 3.5	21.7 ± 1.2	> 100
IC_{90} (μg/mL)	92.4 ± 0.5	> 100	82.5 ± 5.2	46.1 ± 0.8	> 100

Table 5 Cytotoxicity (IC_{50}, IC_{90}) of fractions P1-P15 against HepG2.

Fraction	IC_{50} ± SD (μg/mL)	IC_{90} (μg/mL)	Fraction	IC_{50} ± SD (μg/mL)	IC_{90} ± SD (μg/mL)
P1	> 100	> 100	P9	16.3 ± 2.1	88.9 ± 5.4
P2	86.4 ± 5.3	> 100	P10	15.5 ± 2.9	88.4 ± 2.3
P3	> 100	> 100	P11	24.9 ± 1.1	56.0 ± 11.1
P4	37.5 ± 1.6	> 100	P12	25.0 ± 0.4	49.6 ± 0.1
P5	20.7 ± 1.5	> 100	P13	17.1 ± 1.7	92.0 ± 3.6
P6	15.7 ± 1.3	> 100	P14	20.2 ± 3.9	91.6 ± 2.8
P7	17.8 ± 1.4	> 100	P15	66.4 ± 0.9	95.3 ± 0.7
P8	11.3 ± 0.5	> 100			

α-orientation. The usual H-20β configuration of cycloartane triterpenes was assigned based on the H-18/H-20, H-12/H-21 and H-17/H_3-21 NOESY cross peaks (Nuanyai et al., 2009; Truong et al., 2011; Simo Mpetga et al., 2012).

Compound **2**, a white amorphous powder, had the same molecular formula as **1**. Detailed comparison of Compounds **1** and **2** with euphonerin E indicated that all three shared a planar structure. However, differences in the ^{1}H and ^{13}C chemical shifts of CH-23/CH-24/CH-25/CH_3-27 suggested that the three stereoisomers differed in their C-23 and C-24 configurations. More specifically, the $CDCl_3$ ^{13}C chemical shifts of C-23-C-24-C-25-C-26 in Compound **2** closely matched those of euphonerin E, while those of **1** were clearly shifted [C-23/24/25: 76.4/85.7/141.9 in **1** against 69.5/79.1/144.5 in **2**]. This suggested a *syn* configuration of C-23 and C-24 for **2**, whereas euphonerin E and **1** had the *anti* configuration. An attempt was made to assess the relative configuration of the side chains based on the comparison of the $J_{H\text{-}23/H\text{-}24}$ coupling constant. The tirucallane triterpenoid piscidinol, having *syn*-oriented H-23 and H-24, also had a null coupling constant, while the *anti* epimer–24-*epi*-piscidinol has a coupling constant of $J_{H\text{-}23/H\text{-}24}$ 8.0 Hz, validated by X-ray crystallographic analyses (McChesney et al., 1997). The *syn* and *anti* configurations of C-23 and C-24 in related side chains have similar coupling constant values: 23,24,25-trihydroxycycloartan-3-one ($J_{H\text{-}23/H\text{-}24}$ *ca.* 0 Hz, *syn*) (Joycharat et al., 2008), alisols A and P ($J_{H\text{-}23/H\text{-}24}$ *ca.* 0 Hz, both *syn*) (Nakajima et al., 1994; Zhao et al., 2008), cumingianols A/B ($J_{H\text{-}23/H\text{-}24}$ *ca.* 0 Hz, both *syn*) (Kurimoto et al., 2011), cumingianosides G-J and L-M ($J_{H\text{-}23/H\text{-}24}$ *ca.* 0 Hz, all *syn*) (Fujioka et al., 1997), alisol E ($J_{H\text{-}23/H\text{-}24}$ 6 Hz, *anti*) (Yoshikawa et al.,1993), and toonacilitavarin E ($J_{H\text{-}23/H\text{-}24}$ 7.5 Hz, *anti*) (Zhang et al., 2012). Interestingly, when the classical *gem*-dimethyl substituent is converted to an isopropenyl moiety, the magnitude of the coupling constant appears to change: unnamed argenteanol B derivative ($J_{H\text{-}23/H\text{-}24}$ 6.5 Hz, *syn*) (Mohamad et al., 1997), cumingianol C ($J_{H\text{-}23/H\text{-}24}$ 5.5 Hz, *syn*), cumingianosides D and N ($J_{H\text{-}23/H\text{-}24}$ 6.0 and 5.5 Hz, respectively, *syn*) (Fujioka et al., 1997), alisol G ($J_{H\text{-}23/H\text{-}24}$ 6.5 Hz, *syn*) (Yoshikawa et al.,1993), and euphonerin E ($J_{H\text{-}23/H\text{-}24}$ 6.7 Hz, *syn*) (Toume et al., 2012). As far as we could ascertain, no anti-disposed OH groups on isopentenyl-bearing side chains have been reported. A literature review suggested that *syn* isomers of compounds that share a side chain with compounds **1** and **2** would be expected to have lower $J_{H\text{-}23/H\text{-}24}$ values than the *anti* compounds. The vicinal coupling constant of compound **2** ($J_{H\text{-}23/H\text{-}24}$ 4.5 Hz) is consistent with a *syn* configuration of euphonerin E, reducing the possible candidates for compound **2** to two (**2a** and **2b**) (Fig. 3). In the same way, two candidates, **1a** or **1b**, were proposed for compound **1** (Fig. 3).

The stereochemistry of the side chains of **1** and **2** was determined based on *ab initio* calculation of NMR shifts, and by subsequently assigning a DP4+ probability score (Smith and Goodman, 2010). This technique is increasingly used when assigning stereochemical structure to natural extracts (Duong et al. 2018b, 2018c). The DP4+ probability suggested that isomers **1b** and **2b** were the most likely candidates for compounds **1** and **2**, being assigned a 100% probability (Fig. 2). The suggested configuration **of 1** was (23*S**,24*R**), and that of **2** (23*R**,24*R**). The final elucidation of compounds **1** and **2** as combretones G and H is shown as Fig. 1.

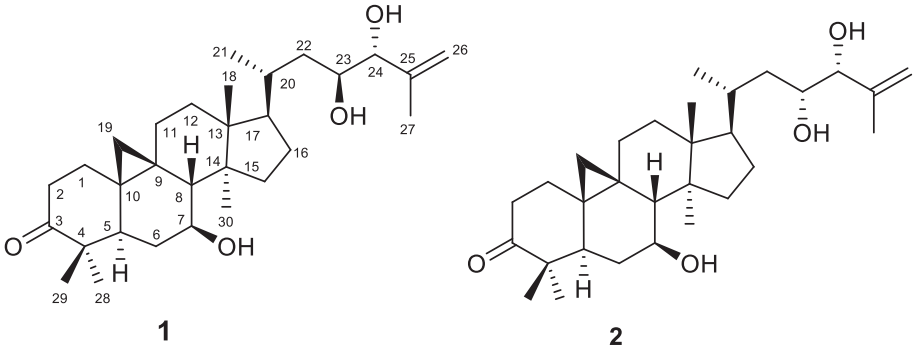

Fig. 1 Structures of compounds **1**–**2**.

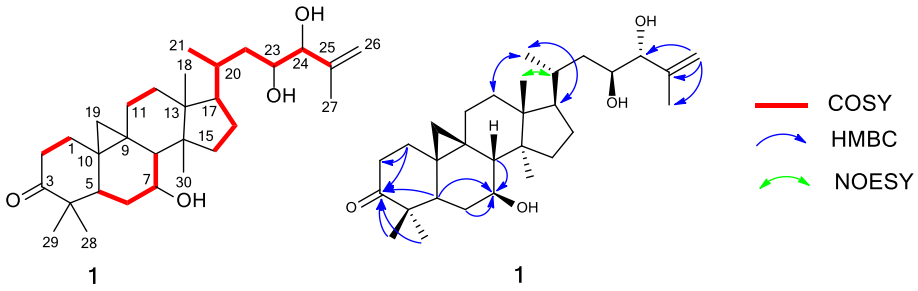

Fig. 2 Key HMBC, COSY, and NOESY correlations of **1**.

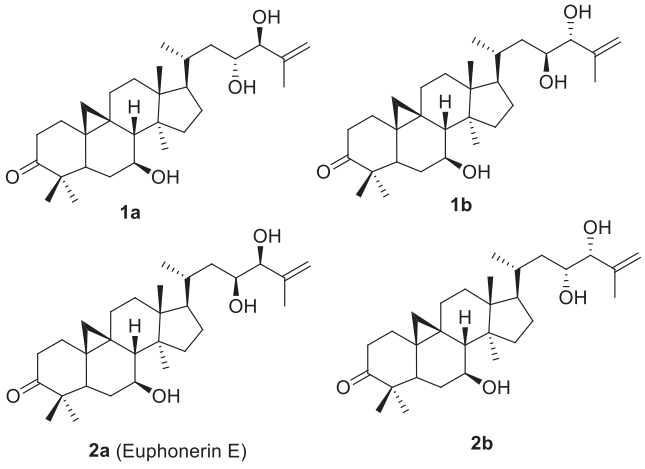

Fig. 3 Four possible isomers of **1** and **2**.

Both compounds were assayed for cytotoxicity against the K562 (chronic myelogenous leukemia), HepG2 (liver hepatocellular carcinoma), and MCF-7 (breast cancer) cell lines. Moderate cytotoxicity was reflected in IC_{50} values of 13.3–70.3 μg/mL (Table 3). Compound **2** showed clear antiparasitic activity against *T. canis* larvae (Table 2) through the low relative mobility suggested dose dependence. At the highest test concentration of 250 μg/mL, 80% of larvae died, giving a mobility score of 0. At the lowest concentration of 5 μg/mL, compound **2** killed 20% of larvae. As the culture medium DMEM had no effect, it was set at 100, corresponding to a mobility score of 3. In DMSO solvent the larvae retained a relative mobility (RM) of 100%, suggesting no -antiparasitic activity. Both albendazole and mebendazole had high RM values, at 3. It was recently reviewed that several natural products (lipids, phenolics, saponin, terpenoids, coumåic acid, miscellaneous) govern anthelmintic activity against nematodes including *Toxocara* spp. *in vitro* and *in vivo* (Liu et al. 2020). However, mechanism under this observation is still controversial. It was shown that several cycloartane triterpenes derived from leaves of *Combretum quadrangulare* and other plants enhanced death receptor 5 (DR5) expression which results in induction of apoptosis in cancer cells (Toume et al., 2011, 2012). Whether *Toxocara* spp. governs DR5 and acts in a similar pathway is unclear. Previous studies documented that the seeds and bark of *Combretum quadrangulare* can be effectively used to remove intestinal helminths in both animals and human (Bui et al., 1978). Our study shows for the first time that leaf extract and compound **2** of *Combretum quadrangulare* expresses anthelmintic activity. We therefore propose compound **2** as a natural antiparasitic that warrants further investigation.

4. Conclusions

Two new cycloartanes, combretanones G and H (**1** and **2**), were isolated from the leaves of *C. quadrangulare*. Their structures were determined by analysis of MS and NMR data and comparison with published values. DFT-NMR chemical shift calculations and DP4/DP4+ probability assignment were used to determine the relative configurations of compounds **1** and **2**. Both exhibited moderate cytotoxicity against K562, HepG2, and MCF-7, with IC_{50} values in the range 13.3–70.3 μg/mL. Compound **2** was shown to be a potent antiparasitic.

Table 6 Cytotoxicity (IC_{50}, IC_{90}) of fractions T1-T8 against HepG2.

Fraction	IC_{50} ± SD (μg/mL)	IC_{90} ± SD (μg/mL)	Fraction	IC_{50} ± SD (μg/mL)	IC_{90} ± SD (μg/mL)
T1	69.7 ± 2.7	> 100	T5	10.1 ± 1.7	42.0 ± 0.7
T2	20.4 ± 7.9	> 100	T6	14.6 ± 0.7	24.7 ± 0.5
T3	18.8 ± 1.4	> 100	T7	14.9 ± 1.1	42.4 ± 3.5
T4	7.0 ± 0.8	34.6 ± 3.4	T8	25.4 ± 1.5	47.5 ± 0.3

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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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2021.103189.

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