Fitoterapia 130 (2018) 198–202

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Four new C-benzyl flavonoids from the fruit of *Uvaria cherrevensis*

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ARTICLE INFO

Keywords:
Uvaria cherrevensis
Annonaceae
Flavonoids
Antiplasmodial activity
Cytotoxic activity

ABSTRACT

The phytochemical investigation of the fruit extracts of *Uvaria cherrevensis* led to the isolation and characterization of four new C-benzyl flavonoids; cherrevenones A-D (**1**–**4**) together with 11 known compounds. The isolated compounds were characterized using spectroscopic techniques. Compounds **1**, **3**, **5** and **11** showed moderate inhibitory activities against the *P. falciparum* strains TM4/8.2 and K1CB1 with IC_{50} values ranging from 21.0 ± 3.10 – 33.7 ± 7.69 and 21.0 ± 5.44 – 43.5 ± 11.9 μM, respectively. Compounds **1**, **2**, **5**, **10** and **11** exhibited strong cytotoxic activities against KB cells with IC_{50} values ranging from 0.60 ± 0.17 – 4.91 ± 2.69 μM which were similar to their cytotoxic activities found against Vero cells, except for compound **5**, which was non-toxic to Vero cells.

1. Introduction

Uvaria cherrevensis, also known as "Nom Meaw Pa" in Thai, with the synonym of *Ellipeiopsis cherrrevensis*, is a monotypic species in the Annonaceae family [1,2]. This plant is a shrub which is distributed throughout the forests of Thailand [1]. The roots of this plant have been used in traditional medicine to treat urinary disorders [3]. Previous phytochemical studies of this plant reported the isolation of alkaloids, flavonoids, naphthalene derivatives, polyoxygenated cyclohexenes and terpenoids [3–6]. Some of these compounds were reported to have antimalarial, antimicrobial and cytotoxic activities [3,5,6]. In a previous report, we found 2-phenylnaphthalene derivatives, polyoxygenated cyclohexenes and flavonoids from the stems and roots extracts of this plant [5]. Herein, we report the results of a phytochemical investigation of the fruit extracts of *U. cherrevensis* which resulted in the isolation and identification of four new C-benzyl flavonoids (**1**–**4**) together with 11 known compounds. The antimalarial and cytotoxic activities of these compounds, against KB and Vero cells, are also reported.

2. Experimental

2.1. General experimental procedures

Melting points were determined on a Stuart SMP10 melting point apparatus and are uncorrected. Optical rotations were measured in acetone at the sodium D-line on a Rudolph Research Analytical Autopol I polarimeter. UV–vis absorption spectra were measured in MeOH with a Thermo Scientific Evolution 210 UV–vis spectrophotometer. The infrared (IR) spectra were recorded on a Bruker Tensor 27 FT-IR spectrophotometer. The NMR spectra were recorded on either a 400 MHz or a 500 MHz Bruker NMR spectrometer. Chemical shifts were recorded in parts per million (δ) in $CDCl_3$ (δ_H 7.26 and δ_C 77.0 ppm) and/or acetone-d_6 (δ_H 2.05 and δ_C (CO) 206.2 and (CH_3) 29.8 ppm) with TMS as internal standard. The HRESIMS data were obtained on a Bruker Daltonics and Thermo Fisher mass spectrometer. Thin-layer chromatography (TLC) was performed on silica gel 60 GF_{254} (Merck). Column chromatography (CC) was carried out on Sephadex LH-20 or silica gel (Merck) type 100 (62–400 μm). Silica gel type 60 (5–40 μm) was used for quick column chromatography (QCC). Solvents for extraction and chromatography were distilled prior to use.

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https://doi.org/10.1016/j.fitote.2018.08.020
Received 2 August 2018; Received in revised form 24 August 2018; Accepted 24 August 2018
Available online 27 August 2018

Table 1
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectroscopic data of cherrevenones A - C (**1–3**) in acetone-d_6.

Position	**1**[a]			**2**			**3**		
	δ_H, (J in Hz)	δ_C, type		δ_H, (J in Hz)	δ_C, type		δ_H, (J in Hz)	δ_C, type	
1		136.4	C		128.1	C		136.4	C
2	7.50 d (7.6)	130.0	CH	7.59 d (8.6)	131.3	CH	7.46 m	129.9	CH
3	7.82 d (7.6)	129.3	CH	6.90 d (8.6)	116.8	CH	7.74 dd (8.0,1.4)	129.2	CH
4	7.48 t (7.6)	131.2	CH		160.8	C	7.45 m	131.1	CH
5	7.82 d (7.6)	129.3	CH	6.90 d (8.6)	116.8	CH	7.74 dd (8.0,1.4)	129.2	CH
6	7.50 d (7.6)	130.0	CH	7.59 d (8.6)	131.3	CH	7.46 m	129.9	CH
7	7.83 d (15.6)	142.9	CH	7.72 d (15.5)	143.6	CH	7.85 d (15.7)	143.4	CH
8	8.16 d (15.6)	128.7	CH	7.90 (15.5)	127.9	CH	8.03 d (15.7)	127.3	CH
9		193.6	C		193.5	C		193.0	C
1′		105.6	C		106.2	C		108.5	C
2′		164.0	C		165.3	C		164.6	C
3′		110.2	C		108.1	C		112.1	C
4′		160.6	C		163.2	C		161.1	C
5′		101.2	C	6.14 br s	92.2	C		115.1	C
6′		152.7	C		162.5	C		160.9	C
7′	3.98 s	22.7	CH_2	3.88 s	22.8	CH_2	3.96 s	23.7	CH_2
8′	3.91 s	22.8	CH_2				3.96 s	24.4	CH_2
1″		127.8	C		127.8	C		128.3	C
2″		153.7	C		155.0	C		155.3	C
3″	6.93 d (7.5)	115.7	CH	6.78 dd (7.6,1.8)	115.9	CH	6.83 dd (7.5,1.6)	116.0	CH
4″	7.07 t (7.5)	128.3	CH	6.97 td (7.6,1.8)	127.9	CH	6.98 td (7.5,1.6)	127.7	CH
5″	6.84 t (7.5)	121.7	CH	6.70 td (7.6,1.8)	120.7	CH	6.75 td (7.5,1.6)	120.8	CH
6″	7.44 d (7.5)	131.9	CH	7.24 dd (7.6,1.8)	131.2	CH	7.36 dd (7.5,1.6)	131.7	CH
1‴		121.2	C					128.1	C
2‴		151.4	C					155.7	C
3‴	7.05 d (7.4)	116.8	CH				6.83 dd (7.9,1.6)	115.9	CH
4‴	7.25 t (7.4)	128.8	CH				7.02 td (7.9,1.6)	128.0	CH
5‴	7.11 t (7.4)	125.0	CH				6.70 td (7.9,1.6)	120.3	CH
6‴	7.30 d (7.4)	130.4	CH				7.08 dd (7.9,1.6)	130.7	CH
2′-OH	14.35 s						14.17 s		
6′-OMe				3.91 s	56.3	CH_3	3.68 s	63.6	CH_3

[a] Measured at 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR).

2.2. Plant material

The fruit of *Uvaria cherrevensis* were collected from Doi Suthep National Park, Chiang Mai, Thailand in August 2015. This plant was identified by Dr. Tanawat Chaowasku from the CMUB Herbarium, Chiang Mai University, Thailand, where a voucher specimen has been deposited (specimen no. T. Ritthiwigrom 5).

2.3. Extraction and isolation

The air dried fruit of *U. cherrevensis* (306.6 g) was extracted with MeOH (3 L) at room temperature over a period of 3 d. Removal of the solvent under reduced pressure afforded a brown viscous oil (37.36 g). The oil was separated by QCC over silica gel. The column was eluted with a solvent gradient from hexane (100%) to acetone (100%) to MeOH (100%) to provide eight fractions (Fractions A-H, see the Supplementary Information for a flow chart of the isolation procedure). Fraction A (654.7 mg) was separated by CC over Sephadex LH-20 with MeOH elution to give four subfractions (A1-A4). Subfraction A2 (19.6 mg) was further purified by CC over silica gel by elution with EtOAc/hexane (15:85) to give compound **5** (10.0 mg) as a yellow viscous oil. Compound **1** (11.0 mg), as a yellow solid, mp 224–226 °C, was obtained from subfraction A4 after evaporation. Fraction B (394.2 mg) was purified by CC over Sephadex LH-20 with MeOH elution to provide compound **6** (1.2 mg) as a yellow solid. Fractionation of Fraction C (1.85 g) by CC over Sephadex LH-20 with MeOH elution gave three subfractions (C1-C3). Subfraction C2 (48.3 mg) was purified by CC over silica gel by elution with EtOAc/hexane (1:4) to give compound **7** (12.7 mg) as a white solid, mp 195–197 °C, lit. 182–183 °C [3]. Fraction E (1.16 g) was separated by CC over silica gel by elution with MeOH/CH_2Cl_2 (1:99) to give ten fractions (E1-E10). Compound **8** (7.5 mg), as a yellow solid, mp 146–147 °C, lit. 179–181 °C [3], was obtained from subfraction E2 (10.4 mg) by CC over Sephadex LH-20 with MeOH elution. Subfraction E4 (285.7 mg) was separated by CC over silica gel by elution with MeOH/CH_2Cl_2 (1:99) to provide compounds **3** (11.5 mg) and **9** (23.9 mg) as a yellow viscous oil and a brown solid (mp 204–205 °C, lit. 203.5–204.5 °C [7]), respectively. Subfraction E6 (46.6 mg) was separated by CC over Sephadex LH-20 with MeOH elution to give four subfractions (E6A-E6D). Subfraction E6B (21.5 mg) was purified by CC over silica gel by elution with MeOH/CH_2Cl_2 (1:99) to provide compound **10** (6.5 mg) as a yellow viscous oil. Compound **11** (10.6 mg), as a yellow viscous oil, was obtained from subfraction E6C after evaporation. Subfraction E8 (46.2 mg) was separated by CC over Sephadex LH-20 with MeOH elution to give three subfractions (E8A-E8C). Subfraction E8B (25.5 mg) was purified by CC over silica gel by elution with MeOH/CH_2Cl_2 (1:99) to give compound **12** (7.4 mg) as a yellow solid, mp 99–100 °C (mp not reported in the literature). Subfraction E9 (47.1 mg) was separated by CC over Sephadex LH-20 with MeOH elution to give compound **13** (2.1 mg) as a brown viscous oil. Fraction F (1.11 g) was separated by CC over Sephadex LH-20 with MeOH elution to give three subfractions (F1-F3). Subfraction F2 (34.3 mg) was separated by CC over silica gel by elution with EtOAc/CH_2Cl_2 (5:95) to provide compound **2** (2.5 mg) as a yellow viscous oil. Fraction H (530.0 mg) was separated by CC over Sephadex LH-20 with MeOH elution to provide four subfractions (H1-H4). Compound **14** (13.5 mg), as a yellow solid, mp 225–227 °C (mp not reported in the literature), was isolated from subfraciton H2 (81.5 mg) by CC over silica gel by elution with MeOH/CH_2Cl_2 (2:98). Subfraction H3 (167.6 mg) was separated by CC over Sephadex LH-20 with MeOH elution to give five subfractions (H3A-H3E). Subfraction H3B (25.5 mg) was further purified by CC over silica gel by elution with MeOH/CH_2Cl_2 (2:98) to give compound **15** (3.5 mg) as a yellow viscous oil. Compound **4** (1.3 mg), as a yellow viscous oil, was obtained from subfraction H3D (13.1 mg) by CC over silica gel by elution with MeOH/CH_2Cl_2 (1:99).