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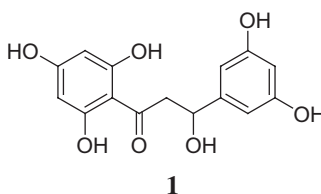
Balanochalcone, a new chalcone from *Balanophora laxiflora* Hemsl.

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ABSTRACT

A new chalcone named as balanochalcone (**1**) together with eight known compounds, methyl caffeate (**2**), β -hydroxydihydrochalcone (**3**), methyl gallate (**4**), dimethyl-6,9,10-trihydroxybenzo[*k*]xanthene-1,2-dicarboxylate (**5**), *p*-coumaric acid (**6**), quercetin (**7**), scopoletin (**8**) and pinoresinol (**9**) have been isolated from the ethyl acetate extract of Vietnamese *Balanophora laxiflora* Hemsl. Their structures were characterised by IR, UV, HR-ESI-MS, 1D and 2D NMR and CD spectroscopies. Compounds **2** and **5** showed moderate cytotoxicity against four cancer cell lines, KB (a human epidermal carcinoma), MCF7 (human breast carcinoma), SK-LU-1 (human lung carcinoma) and HepG2 (hepatocellular carcinoma). In addition, compounds **1** and **5** showed moderate antioxidant activity.



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1. Introduction

The genus *Balanophora* (Balanophoraceae family) currently comprises about 120 species in our globe. In Vietnam, *Balanophora laxiflora* Hemsl. is a dioeciously parasitic plant, mainly distributed in the forests in Hoa Binh, Lao Cai, Yen Bai provinces. The whole plant of *B. laxiflora*

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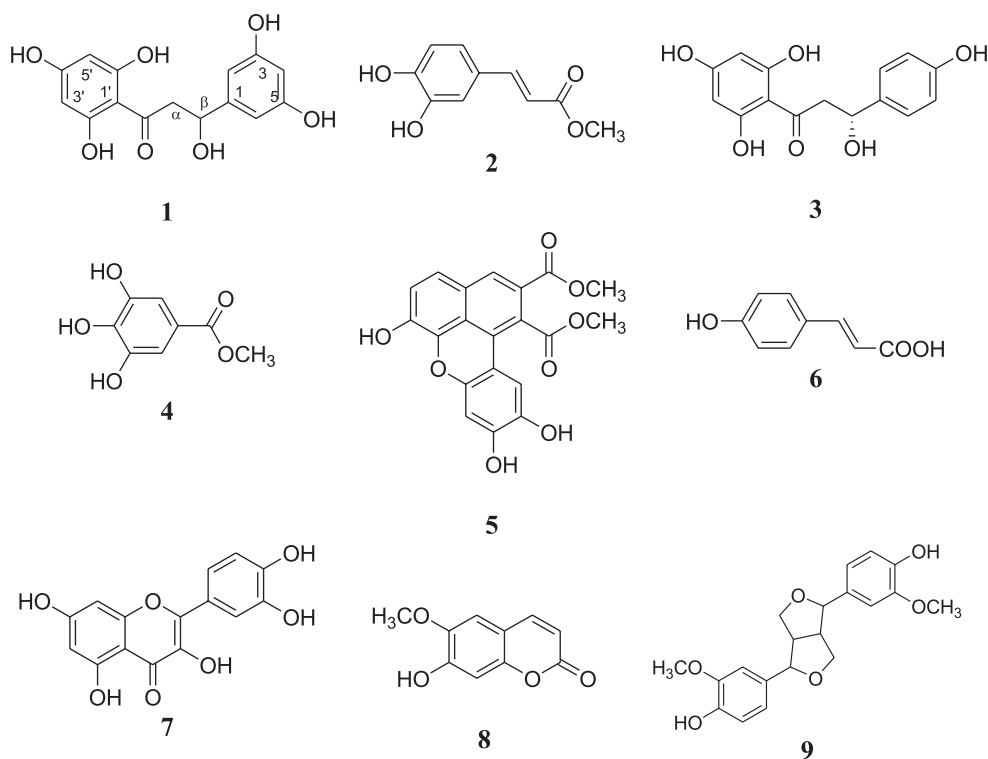


Figure 1. Chemical structures of compounds 1–9 isolated from *B. laxiflora*.

has been used as a tonic for blood circulation improvement, recovery, antipyretic, antidote, appetite stimulation (Do 2015). Recent researchers have discovered various compounds and bioactivities of *B. laxiflora*. For instance, antioxidant hydrolysable tannins with a phenylacrylic acid derivative such as caffeoyl, coumaroyl, linked to C-1 of a glucosyl unit by *O*-glycosidic bond (She et al. 2009; Ho et al. 2010; Wang et al. 2012); anti-inflammatory metabolites (Chiou et al. 2011), hypouricemic activity (Ho et al. 2012). In order to elucidate biochemical and bioactive significance as well as extend the use of *B. laxiflora*, this paper reports our recent chemical investigation on the ethyl acetate extract of *B. laxiflora*, collected in Lao Cai province together with the cytotoxicity and antioxidant activities.

2. Results and discussion

The EtOAc extract of the *B. laxiflora* was subjected repeatedly to silica gel and Sephadex LH-20 column chromatography, followed by preparative HPLC to yield nine compounds (1–9) (Figure 1).

Compound 1 was obtained as an oil. The molecular formula was established as $C_{15}H_{14}O_7$ by positive HR-ESI-MS, which showed a *quasi*-molecular ion peak $[M + H - H_2O]^+$ at 289.0696 (Calcd for $C_{15}H_{13}O_6$, 289.0712). Its UV spectrum absorption maxima of 225 and 288 nm together with IR absorption bands for hydroxyl (3200 cm^{-1}), carbonyl (1633 cm^{-1}) and aromatic rings (1601 and 1530 cm^{-1}) revealed the β -hydroxydihydrochalcone skeleton for compound 1 (Muiva et al. 2009; Özbek et al. 2016). Then, the structure of 1 was deduced from

analysis of its 1D and 2D NMR spectra. Its ^1H NMR spectrum shows the presence of five olefinic protons, one carbinol and one methylene group. Analysis of its ^{13}C NMR spectrum indicates the presence of 15 carbon signals, including one conjugated ketone (197.8 ppm), two aromatic rings. Three singlet protons resonanced at 6.81 (2H) and 6.94 (1H) ppm are assigned at C-2, C-4 and C-6 of the first aromatic ring due to the HBMBC correlations between (1) H-2 and H-6/C-1, C-3, C-4 and C-5; (2) H-4/ C-1, C-2, C-3, C-5. This ring is connected to C- β is proved by the long-range correlations from C- β to H-2, H-6. In addition, two doublet protons at 5.92 and 5.90 ppm which both have a small coupling constant ($J = 2.0$ Hz), therefore, they are characterised at *meta*-position (H-3' and H5') of the second ring. The hydroxyl group is placed at C- β due to its low field shift in ^{13}C NMR spectrum and HMBC correlation from (i) H- β to C=O; (ii) H- α to C- β , C=O, C-1 (Muiva et al. 2009; Özbek et al. 2016). From above discussion, compound **1** is found to be a new β -hydroxydihydrochalcone and given a trivial name as balanochalcone. This appears to be the first report on the occurrence of a β -hydroxydihydrochalcone in the *B. laxiflora*. The structures of the rest eight compounds have been characterised by comparison with the literature data as methyl caffeate (**2**) (Xiang et al. 2011), β -hydroxydihydrochalcone (**3**) (Tanaka et al. 2007), methyl gallate (**4**) (Ekaprasada et al. 2009), dimethyl 6,9,10-trihydroxybenzo[*k*]xanthene-1,2-dicarboxylate (**5**) (Daquino et al. 2009), *p*-coumaric acid (**6**) (Durust et al. 2001), quercetin (**7**) (Huang et al. 2013), scopoletin (**8**) (Darmawan et al. 2012) and pinoresinol (**9**) (Owen et al. 2000). However, the absolute configuration of the C- β of compound **1** was failed to be determined. Since, its optical rotation have almost a zero degree, in addition, no cotton effect was observed from it CD spectrum.

Previous investigations have shown that β -hydroxydihydrochalcone possessing valuable biological activity such as antioxidant (Özbek et al. 2016), antiplasmodial activity (Muiva et al. 2009). Then, the cytotoxicity of **1–3** and **5** were tested against four cancer cell lines as illustrated in Table 1. Accordingly, compounds **2** and **5** showed good and non-selective activity. The antioxidative activity of **1** and **5** was also estimated by an indication of a 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging effect (Blois 1958). Compounds **1** and **5** showed moderate activity as compared with ascorbic acid (IC_{50} : 18.42 ± 1.64 $\mu\text{g/mL}$) with the IC_{50} values of 65.10 ± 2.58 and 76.74 ± 3.06 $\mu\text{g/mL}$, respectively.

3. Experimental

3.1. General

IR spectra were recorded on a Thermo Nicolet 5700 FT-IR spectrometer. UV spectra were recorded on a Jasco V-560 spectrophotometer. CD spectra were measured on a Chirascan, Applied Photophysics Ltd., Surrey, United Kingdom in methanol. NMR spectra were recorded on a Bruker Avance 500 MHz. The chemical shift (δ) values are given in ppm with TMS as

Table 1. Cytotoxic activity of compounds **1–3**, **5** isolated from *B. laxiflora* (IC_{50} , $\mu\text{g/mL}$).

Compounds/cells	KB	Hep-G2	Lu-1	MCF-7
Compound 1	> 64	> 64	> 64	> 64
Compound 2	20.77	43.75	106.05	87.17
Compound 3	59.9	> 128	> 128	> 128
Compound 5	30.7	55.9	55.4	68.02
Ellipticin (Standard)	0.31	0.35	0.45	0.53

internal standard, coupling constant J (by Hz). High-resolution ESI MS were measured on a Bruker Apex III Fourier transform ion cyclotron resonance (FTICR) mass spectrometer. Silica gel (Merck Co., Germany) was used for flash chromatography. Preparative HPLC was performed on a Jasco PU-2087 instrument with a UV-2070 and RI-2031 detectors using a Waters 5C 18-AR-II (10.0 × 250 mm), flow rate of 1.0 mL/min. TLC was carried out on pre-coated Si gel GF₂₅₄ (Merck Co., Germany) and TLC spots were viewed at 254, 302 and 366 nm and visualised by spraying with vanillin- 10% H₂SO₄ solution.

3.2. Plant material

The whole parts of *Balanophora laxiphora* plant were collected in Lao Cai province (October, 2014). The plant material was identified by Dr Do Huu Thu, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, Hanoi, Vietnam. The voucher specimens (DNQ-2014NC) were deposited at the Herbarium of Hanoi National University of Education, Hanoi, Vietnam.

3.3. Extraction and isolation

The fresh *B. laxiflora* (4.0 kg) were extracted with 80% MeOH (10 L × 3) at room temperature and evaporated all solvent to give a crude extract (320 g). The crude extract was subjected to fractional extraction and, then, vacuum evaporation, giving *n*-hexane (53.4 g), ethyl acetate (28.0 g), butanol (123.3 g) and methanol (80.2 g) extracts, respectively. The ethyl acetate extract (28.0 g) was further subjected to silica gel column chromatography, using *n*-hexane/EtOAc to give 10 fractions. Fraction 7 (1.64 g) was chromatographed on silica gel column, eluting with CHCl₃/*n*-hexane (98/2) to give **2** (20.9 mg) and a mixture (0.31 g) which was purified by Sephadex LH-20, CHCl₃/MeOH (2/1) followed by prep. HPLC with RP-18 column, MeOH/H₂O (1/1) to afford **4** (1.6 mg), **3** (28.1 mg), **5** (5.3 mg) and **6** (4.2 mg). Fraction 8 (5.0 g) was isolated by Sephadex LH-20 column, MeOH/CHCl₃ (3/1) to yield four sub-fractions. Sub-fr. 1 (848 mg) and sub-fr. 3 (109.1 mg) were purified by silica gel column, *n*-hexane/acetone (3/1) to obtain **8** (6.7 mg) and **9** (121.7 mg) and **1** (6.7 mg). Sub-fr. 4 (297.1 mg) was purified by prep. HPLC, using MeOH/H₂O (6/4) to give **7** (6.3 mg).

Compound **1**: Light yellow oil; UV (MeOH), λ_{\max} nm (log ϵ): 225 (2.19), 288 (2.16); IR (KBr), ν_{\max} cm⁻¹: 3200, 2923, 2852, 1633, 1601, 1530, 1454, 1159; ¹H NMR (500 MHz, CD₃OD): δ 6.94 (1H, s, H-4), 6.81 (2H, s, H-2 and H-6), 5.92 (1H, d, J = 2.0 Hz, H-5'), 5.90 (1H, d, J = 2.0 Hz, H-3'), 5.34 (1H, dd, J = 3.0, 7.5 Hz, H- β), 3.90 (1H, dd, J = 7.5, 17.0 Hz, H- α), 3.72 (1H, dd, J = 3.0, 17.0 Hz, H- α); ¹³C NMR (125 MHz, CD₃OD-*d*₄): δ 197.8 (C = O), 168.4 (C-4'), 165.5 (C-6'), 164.8 (C-2'), 146.9 (C-3), 146.5 (C-5), 131.8 (C-1), 119.3 (C-6), 116.3 (C-2), 114.7 (C-4), 103.4 (C-1'), 97.0 (C-3'), 96.2 (C-5'), 80.5 (C- β), 44.1 (C- α); HR-ESI-MS, m/z : 289.0696 [M + H - H₂O]⁺ (Calcd for C₁₅H₁₃O₆, 289.0712).

3.4. Bioassays

Cytotoxic assays of compounds **1–3** and **5** were tested against four cancer cell lines from an American Type Culture Collection according to the method described by (Scudiero et al. 1988). Cell lines were cultured in an RPMI 1640 medium supplemented with a 10% foetal bovine serum (FBS) in standard condition, sterile with 5% CO₂ at 37 °C, 98% humidity and

harvested at log phase for assays. In this assay, 200 μL volumes of cells at a concentration of 3×10^4 cells mL^{-1} were inoculated into a 96-well plate in a RPMI 1640 medium. Compounds **1–3** and **5** were applied at final concentrations 128, 32, 8, 2 and $0.5 \mu\text{L}^{-1}$ and the cultures were incubated for three days at 37°C with 5% CO_2 . Then, 50 μL of MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, prepared at 1 mg mL^{-1} in FBS, was added to the microculture. After 4 h of incubation, 250 μL of the supernatant was removed from each well and 100 μL of DMSO was added, mixing thoroughly. Absorbance was measured at 540 nm in a Genios TECAN spectrophotometer. The IC_{50} value was calculated based on per cent growth inhibition $(\text{OD}_{\text{control}} - \text{OD}_{\text{sample}})/\text{OD}_{\text{control}}$.

Antioxidant activity of **1** and **5** was evaluated by determining free radical-scavenging potential using DPPH according to Blois 1958. The reaction mixture contained 20 μL of extract solutions and 180 μL of 0.1 mM DPPH solution. Ascorbic acid was used for comparison with extracts. DPPH scavenging activity was calculated using the following formula: DPPH scavenging activity (%) = $[(A_{\text{control}} - A_{\text{sample}})/(A_{\text{control}})] \times 100$. Where A_{control} represents the absorbance of the control and A_{sample} is the absorbance of the test sample. The IC_{50} value is deduced from the logarithm curve of scavenging capacity vs. sample concentration.

4. Conclusions

Phytochemical studies on the ethyl acetate extract of Vietnamese *B. laxiflora* Hemsl. resulted in the isolation of a new chalcone named as balanochalcone (**1**) together with eight known compounds, methyl caffeate (**2**), β -hydroxydihydrochalcone (**3**), methyl gallate (**4**), dimethyl 6,9,10-trihydroxybenzo[*kl*]xanthene-1,2-dicarboxylate (**5**), *p*-coumaric acid (**6**), quercetin (**7**), scopoletin (**8**) and pinoresinol (**9**). Compounds **2** and **5** show moderate cytotoxicity against all four cancer cell lines. Furthermore, compounds **1** and **5** also show moderate antioxidant activities.

Supplementary material

Supplementary material (1D NMR, 2D NMR, IR, UV, CD and HRMS spectra of compound **1**) associated with the article could be found on the internet in the online version.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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