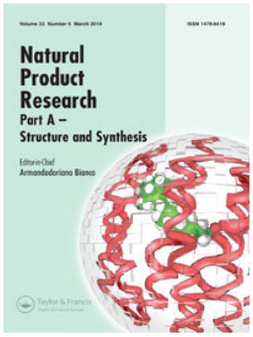

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# A new formylated chalcone from *Humulus lupulus* with protective effect on HUVECs injury by angiotensin II

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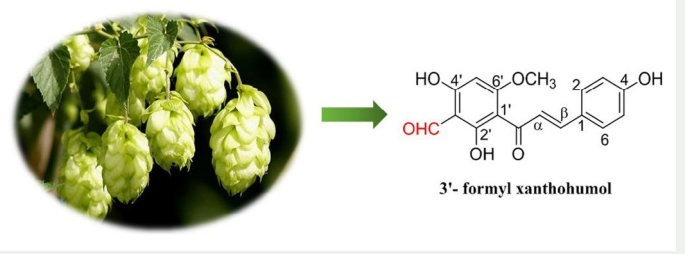
**ABSTRACT**

One new formylated chalcone, 3′- formyl xanthohumol (**1**) was isolated from the EtOAc-soluble partition of the cones of *Humulus lupulus*, along with two other known chalcones, namely dehydrocycloxanthohumol (**2**) and xanthohumol (**3**). The structure of compound **1** was elucidated on the basis of its 1D, 2D NMR and MS data. The structures of the known compounds were identified by comparison of their spectroscopic data with those reported by the literatures. The isolates were tested for their protective effects on human umbilical vein endothelial cells (HUVECs) injured by angiotensin II (Ang II), all the three compounds protected against the cell injury at the concentration of 20 μM, and compound **3** showed the most potent activity by improving cell viability from 53.9 to 74.9%.




3'- formyl xanthohumol

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**KEYWORDS**
*Humulus lupulus*; *C*-formylated chalcone; angiotensin II; HUVECs injury

## 1. Introduction

*Humulus lupulus* belongs to Cannabaceae family, its female inflorescences, also called hops have been used in brewing industry to add bitterness and flavour to beer. Multiple biological activities of hops, such as anti-inflammatory (Bohr et al. 2005), anticancer (Wang et al. 2012), antiatherogenic (Hirata et al. 2012), anti-microbial activities (Schurr et al. 2015), anti-obesity (Wilson and Grundmann 2017) and protective effects against post-menopausal bone loss (Keiler et al. 2013), have recently drawn increasing interest of many researchers to their

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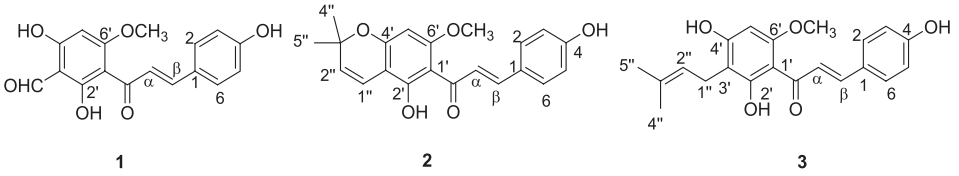
pharmaceutical use. It has been reported that prenylated chalcones and phloroglucinol derivatives (iso-α-acids, α-acid and β-acids) are the main constituents of hops (Karabin et al. 2015; Steenackers et al. 2015). In our on-going study of bioactive compounds from hops, one new chalcone, named 3′-formyl xanthohumol (**1**), together with another two known chalcone-type compounds, named dehydrocycloxanthohumol (**2**) and xanthohumol (**3**) were isolated from the EtOAc partition of the ethanol extract of the cones of *H. lupulus*. Herein, we describe the isolation, structural elucidation of **1** and protective effects of **1**–**3** on human umbilical vein endothelial cells (HUVECs) injured by angiotensin II (Ang II).

## 2. Results and discussion

Compound **1** was obtained as orange needles in MeOH and its molecular formula was $C_{17}H_{14}O_6$ deduced from the positive HR-ESI (*m/z* 337.0716 $[M + Na]^+$, calcd for 337.0683), indicating 11 degrees of unsaturation. The $^1$H NMR spectrum of the compound showed the presence of two benzene rings [$\delta_H$ 7.59 (2H, d, *J* = 8.6 Hz, H-2, 6), 6.84 (2H, d, *J* = 8.6 Hz, H-3, 5), 6.17 (1H, s, H-5′)] and a methoxy group [$\delta_H$ 3.95 (3H, s), $\delta_C$ 56.8]. The *trans*-coupling olefinic protons of $^1$H NMR at $\delta$ 7.38 (1H, d, *J* = 15.7 Hz, H-α) and 7.60 (1H, d, *J* = 15.7, H-β) combined with $^{13}$C NMR signals of $\delta_C$ 123.5 (C-α), 144.9 (C-β) and 191.9 (C=O) and the resonances of two benzene rings suggested the skeleton of the compound was a chalcone, which covered 10 degrees of unsaturation. The signals of $\delta_H$ 10.11 (1H, s, –CHO) and $\delta_C$ 192.0 (C–CHO) evidenced for the presence of a formyl group, which filled the left one unsaturation degree. The coupling and integrations of the two signals at $\delta_H$ 7.59 (2H, d, *J* = 8.6 Hz, H-2, 6) and 6.84 (2H, d, *J* = 8.6 Hz, H-3, 5) suggested the B-ring was substituted at position C-4. Though the signals of hydroxy groups were not observed on $^1$HNMR spectrum, the signals of oxygenated carbons ($\delta_C$ 160.4, 167.6, 167.7) and molecular weight of 314 indicated the presence of three hydroxy groups. The data of $^1$H and $^{13}$C NMR spectra were similar to those of xanthohumol reported by the literature (Stevens et al. 1997), except for the missing resonances of an isoprenyl moiety and the appearance of signals as a formyl group. The position of the formyl group was deduced by the main correlations from HMBC spectra (see supporting material) as follows: H–CHO [$\delta_H$ 10.11 (1H, s)] with C-2′ ($\delta_C$ 167.6) and C-4′ ($\delta_C$ 167.6); H-5′ [$\delta_H$ 6.17 (1H, s)] with C-1′ ($\delta_C$ 105.7) and C-3′ ($\delta_C$ 104.7), and all of the main signals were assigned. Thus, the structure of compound **1** was characterised as a new *C*-formylated chalcone shown in Figure 1 and named as 3′-formyl xanthohumol.

The known compounds **2** and **3** were determined by comparison of their spectral data with those reported in literatures (Stevens et al. 1997; Chen et al. 2012).

Chalcones are widespread in fruits, vegetables, spices, tea and soy-based foodstuff. Chemically, chalcone-type compounds, considered as open-chain flavonoids in which the




**1** **2** **3**

**Figure 1.** Structures of compounds **1**–**3**.

two aromatic rings are linked by a three-carbon α, β-unsaturated system, have a common scaffold of 1,3-diaryl-2-propen-1-one (Matos et al. 2015), the benzene rings of chalcones are substituted most frequently by hydroxy groups followed by methyl, prenyl, isoprenyl and methoxy groups, while formyl substitution is rarely seen [among 59 chalcones listed in literature (Zhuang et al. 2017), only one is substituted by formyl group]. 3′-formyl xanthohumol is the first formyl-substituted chalcone discovered in the genus Humulus.

The isolated compounds **1**–**3** were evaluated by MTT assay for their protective effects against HUVECs injury caused by Ang II, they all could raise the cell viability at the concentration of 20 μM (Figure 2), and compound **3** showed the most potent activity by improving cell viability from 53.9 to 74.9%.

## 3. Experimental

### 3.1. General experimental procedures

NMR spectra were recorded on a Bruker AM-400 NMR instrument with TMS as internal standard. ESIMS data were recorded on an Agilent 6538 Q-TOF mass spectrometer (Agilent Technologies, Waldbronn, Germany). Silica gel (200-300 mesh, Qingdao Marine Chemical Inc., Qingdao/China) and Sephadex LH-20 (Pharmacia Fine Chemical Co. Ltd., Germany.) were used for column chromatography.

### 3.2. Plant material

The female inflorescences of *H. lupulus* (cones of hops) were collected from Inner Mongolia, China in 2012. The plant material was identified by Associate Prof. Qingde Long from Department of Pharmacognosy. A voucher specimen (No. 201009) was deposited at the herbarium of School of Pharmacy, Guizhou Medical University.

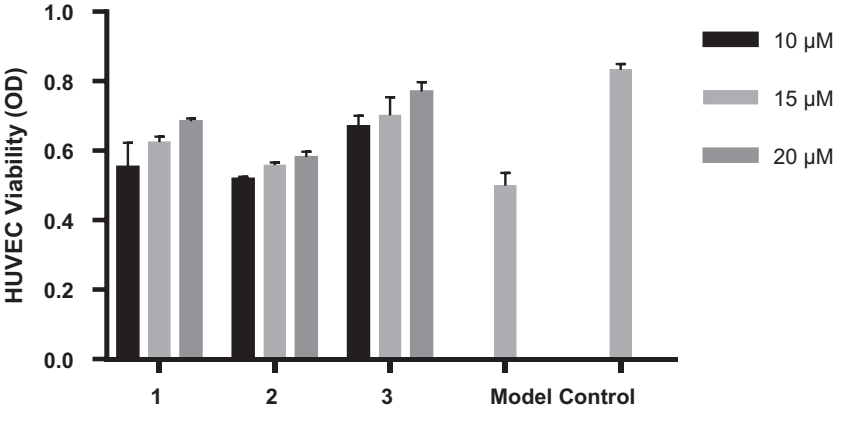



**Figure 2.** Protective effects of chalcones (compounds **1**–**3**) on HUVECs injured by 5 μM Ang II. In the MTT assay, HUVECs were pre-treated with Ang II and concentrations of compounds **1**–**3** (10–20 μM), Ang II alone group was used as model and group with PBS were employed as control. Values are expressed as means ± SD, $n = 3$. All the data except that of **1** and **2** at 10 μM are significantly different from model ($p < 0.01$).

### 3.3. Extraction and isolation

The dried female inflorescences of *H. lupulus* (10 kg) were powdered and extracted with 95% ethanol (20 L × 3) at room temperature, for three days each. The extract was concentrated under *vacuum* to give a residue which was suspended in water and then partitioned successively with petroleum ether, EtOAc and *n*-BuOH. The EtOAc-soluble extract (200 g) was subjected to a silica gel column chromatography (CC) and eluted with $CHCl_3$-MeOH mixtures with gradient elution of $CHCl_3$/MeOH (100:0–70:30, *v/v*) to yield six fractions (Fr.A–Fr.F). Fr.D (20 g) was subjected to silica gel CC using petroleum ether-EtOAc (90:10–60:40) to obtain five sub-fractions (Fr.D1–Fr.D5), red needle crystals were found in the long deposited Fr.D4 (dissolved in MeOH) and washed by $CH_2Cl_2$ to afford compound **1** (8 mg). Fr. D2 and Fr. D3 were further purified by Sephadex LH-20 CC (eluted with MeOH) to give compounds **2** (30 mg) and **3** (85 mg). Purity of all the isolated chalcones for MTT assay was >92% detected by HPLC.

#### 3.3.1. 3′- formyl xanthohumol (1)

Orange needle crystal; MS-ESI$^-$ *m/z*: 313 [M-H]$^-$; MS-ESI$^+$ *m/z*: 337 [M + Na]$^+$; HRMS-ESI$^-$ *m/z*: 313.0716 (Calcd 314.0785 for $C_{17}H_{14}O_6$, [M]$^+$);$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.11 (1H, s, –CHO), 7.60 (1H, d, $J$ = 15.7, H-β), 7.59 (2H, d, $J$ = 8.6 Hz, H-2, 6), 7.38 (1H, d, $J$ = 15.7 Hz, H-α), 6.84 (2H, d, $J$ = 8.6 Hz, H-3, 5), 6.17 (1H, s, H-5′), 3.95 (3H, s, –$OCH_3$); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 105.7 (C-1′), 167.6 (C-2′), 104.8 (C-3′), 167.7 (C-4′), 91.3 (C-5′), 166.9 (C-6′), 123.5 (C-α), 144.9 (C-β), 125.5 (C-1), 131.0 (C-2, 6), 116.0 (C-3, 5), 160.4 (C-4), 191.9 (C = O), 192.0 (–CHO), 56.8 (–$OCH_3$).

### 3.4. MTT assay

HUVECs were pre-treated with different concentrations of chalcones (10, 15 and 20 μM, each in triplicate) for 24 h and followed by treatment with Ang II (5 μM) for another 24 h in 96-well plate, except some wells were added with only 100 μL of culture medium as a background well. The medium was discarded and the cells were washed twice with phosphate-buffered solution (PBS), then 20 μL of 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2-H-tetrazolium bromide (MTT, 5 g/L) and the fresh medium were added to each well. After additional incubation at 37° for 4 h, the medium was discarded again and the formazan crystals produced by viable cells were dissolved in DMSO (150 μL). The optical density (OD) was measured at 490 nm using an enzyme-linked immunosorbent assay and cell viability was calculated from the OD of each well. The assay was performed in triplicate, and the data were presented as mean ± SD.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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