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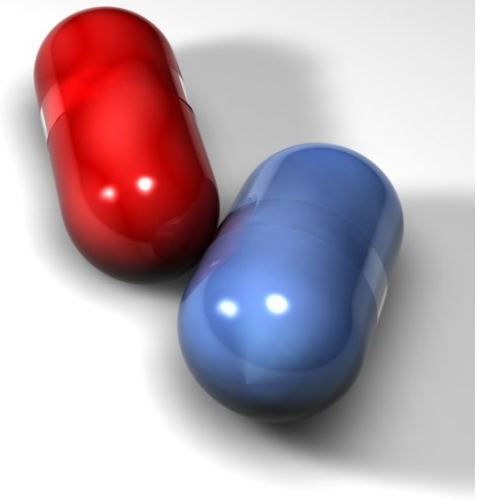

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Letter to the Editor

Cytotoxic phenolic constituents from the leaves of *Ehretia asperula*

Sir,

The genus *Ehretia* is mainly distributes in tropical areas of Asia, Africa, Northern America and exhibited valuable pharmacologial properties (Li et al., 2010; Shukla and Kaur, 2018). In Vietnam, *Ehretia asperula* Zoll. & Mort. has been used in traditional medicine for the treatment of ulcer, tumors, liver disease and inflammation (Nguyen et al., 2017). To date, there are few reports about the biological activities and chemical composition of this plant.

In the present study, the leaves of *E. asperula* were collected from the Bai Rong Hamlet, Thuong Tien Commune, Kim Boi District, Hoa Binh Province. The air-dried and powdered material (5.0 kg) was extracted in methanol at room temperature for 3 days and repeated 3 times. The combined extracts were evaporated under reduced pressure to obtain a crude residue (320 g), which was then re-dissolved in water (2 L) and successively extracted with *n*-hexane and ethyl acetate, respectively. The organic layers were dried in vaccuo to give *n*-hexane and 34.4 g ethyl acetate residues, respectively. Ethyl acetate was chromatographed on a silica gel column eluted with solvent mixtures of dichloromethane and methanol (100/1, 50/1, 10/1, 5/1, 1/1 and 1/100, v/v) to give 6 fractions F1-F6, respectively. The fraction F2 was subjected to a silica gel column eluted with dichloromethane-methanol (5/1) to afford two subfractions F2.1 and F2.2). Subfraction F2.1 was separated on a silica gel column, eluting with *n*-hexane-dichloromethane-methanol (5/15/1, v/v/v) to obtain compound **1** (7.1 mg) and caffeic acid (**2**) (37.5 mg). Methyl caffeate (**3**) (5.0 mg) was purified from subfraction F2.2 by a C_{18} column using methanol-water (1/2, v/v). Fraction F3 was subjected to fractionation over silica gel column, eluted with dichloromethane-acetone (10/1, v/v) to give three subfractions F3.1-F3.3. Subfraction F3.1 was separated using silica gel column and further purified with C_{18} column to yield methyl rosmarinate (**6**) (3.2 mg), oresbiusin B (**7**) (8.1 mg) and dimethyl lithospermate (**8**) (100 mg). Subfraction F3.2 was isolated over silica gel column eluted with dichloromethane-methanol(2:1, v/v) to provide rosmarinic acid (**5**) (3.2 mg). Astragalin (**4**) (11.0 mg) was isolated from subfraction F3.3 using C-18 column chromatography eluted with methanol-water (1/1, v/v).

Compound **1** was isolated as a white amorphous powder and had the molecular formula $C_{15}H_{14}O_4$, as determined by the HR-ESI-MS molecular ion peak at *m/z* 259.0975 $[M+H]^+$. The ^1H NMR spectrum of **1** showed an aromatic ABX spin system at δ_H 7.04 (1H, d, J = 8.0 Hz, H-5), 7.32 (1H, d, J = 2.0 Hz, H-2) and 7.34 (1H, dd, J = 8.0, 2.0 Hz, H-6), an aromatic A_2B_2 protons at δ_H 7.15 (2H, d, J = 8.0 Hz, H-3′, 5′) and 6.84 (2H, d, J = 8.0 Hz, H -2′, 6′). In addition, an aldehyde proton at δ_H 9.71 (1H, s, H-7) and two methylene signals at δ_H 2.73 (2H, t, J = 7.0 Hz, H-7′), 3.71 (2H, t, J = 7.0 Hz, H-8′) were also recognized. The ^{13}C-NMR combined with the HSQC experiments indicated the presence of two aromatic rings [δ_C 131.1 (C, C-1), 118.3 (CH, C-2), 147.2 (C, C-3), 151.8 (C, C-4), 116.1 (CH, C-5), 128.8 (CH, C-6), 158.5 (C, C-1′), 119.5 (CH, C-2′, 6′), 131.0 (CH, C-3′, 5′), 133.7 (C, C -4′)], an aldehyde group [δ_C 193.1 (CH, C-7)] and a 2-subsituted ethanol moiety [δ_C 39.4 (CH_2, C-7′), 64.0 (CH_2, C-8′)]. The HMBC correlations showed the coupling from H-7 to C-1, C-2 and C-6, from H-2 and H -6 to C-7 suggesting the aldehyde group attached to C-1 of the ABX aromatic ring. These data were similar to those of 3,4-dihydroxybenzadehyde (protocatechuic aldehyde) moiety (Ooike et al., 1997). The methylene protons H-7′ coupled to C-4′, C-3′ and C-5′ indicated the ethanol moiety attached to C-3′ of the A_2B_2 aromatic ring, which suggested the structure of 4-(2-hydroxyethyl) -phenol (tyrosol) moiety (Hussain et al., 2014). Based on these data, the structure of **1** consisted of protocatechuic aldehyde and tyrosol fragments. The NOESY spectrum showed the cross peak between H-2 and H-2′, 6′ confirming that two fragments linked via C-3 and C-1' positions (Figure 1). Thus, compound **1** was newly elucidated to be 4-hydroxy-3-[4-(2-hydroxyethyl)-phenoxy]-benzaldehyde.

Cytotoxicity of the isolated compounds was evaluated against human lung (Hep-G2, LU-1), cervical (HeLa), breast (MCF-7), and rhabdomyosarcoma (RD) cancer cell lines (Table I). Compound **1** exhibited the strongest effect to Hep-G2, LU-1, HeLa and RD cells with IC_{50} values in the range of 7.1-10.2 µM. Compounds **2**, **6** and **7** showed moderate activity while **3**, **4**, **5** and **8** were inactive. Interestingly, all compounds did not affect the viability of normal cells (monkey kidney VERO cell line) up to 30 µM. Methyl caffeate (**2**) has been reported as a promising anti-cancer agent by inducing apoptosis (Balachandran et al., 2015; Kim et al., 2019). In this study, compound **1** showed stronger cytotoxic effect than methyl caffeate. Further studies are needed to clarify the anti-cancer ability of *Ehretia asperula* as well as compound **1**.

Table I

Cytotoxicity (μM) of the isolated compounds

Compounds	Hep-G2	LU-1	HeLa	MCF-7	RD	Vero
1	7.1 ± 0.7	8.5 ± 1.1	10.2 ± 0.9	>30	7.9 ± 0.8	>30
2	14.5 ± 2.0	>30	17.4 ± 2.16	22.6 ± 2.69	>30	>30
6	22.1 ± 3.6	>30	24.9 ± 2.63	15.1 ± 1.23	>30	>30
7	25.4 ± 3.5	>30	>30	>30	>30	>30
Ellipticine	3.2 ± 0.2	3.0 ± 0.4	1.3 ± 0.1	4.00 ± 0.4	3.3 ± 0.3	>30

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