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Identification of Soluble Epoxide Hydrolase Inhibitors from the Seeds of *Passiflora edulis* Cultivated in Vietnam

To Dao Cuong[1,2,3,*], Hoang Thi Ngoc Anh[1], Tran Thu Huong[1], Pham Ngoc Khanh[1], Vu Thi Ha[1], Tran Manh Hung[5], Young Ho Kim[6], and Nguyen Manh Cuong[1,4,*]

[1]*Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam*
[2]*Faculty of Pharmacy, Phenikaa University, Yen Nghia, Ha Dong, Hanoi 12116, Vietnam*
[3]*Phenikaa Research and Technology Institute (PRATI), A&A Green Phoenix Group JSC, No.167 Hoang Ngan, Trung Hoa, Cau Giay, Hanoi 11313, Vietnam*
[4]*Graduate University of Science and Technology, Vietnam Academy of Science and Technology, Hanoi, 100803, Vietnam*
[5]*Department of Biomedical Sciences, Institute for Research and Executive Education (VNUK), The University of Danang, 158A Le Loi Street, Hai Chau district, Da Nang 551000, Vietnam*
[6]*College of Pharmacy, Chungnam National University, Daejeon 34134, Republic of Korea*

Abstract – Soluble epoxide hydrolases (sEH) are enzymes present in all living organisms, metabolize epoxy fatty acids to 1,2-diols. sEH in the metabolism of polyunsaturated fatty acids plays a key role in inflammation. In addition, the endogenous lipid mediators in cardiovascular disease are also broken down to diols by the action of sEH that enhanced cardiovascular protection. In this study, sEH inhibitory guided fractionation led to the isolation of five phenolic compounds *trans*-resveratrol (**1**), *trans*-piceatannol (**2**), sulfuretin (**3**), (+)-balanophonin (**4**), and cassigarol E (**5**) from the ethanol extract of the seeds of *Passiflora edulis* Sims cultivated in Vietnam. The chemical structures of isolated compounds were determined by the interpretation of NMR spectral data, mass spectra, and comparison with data from the literature. The soluble epoxide hydrolase (sEH) inhibitory activity of isolated compounds was evaluated. Among them, *trans*-piceatannol (**2**) showed the most potent inhibitory activity on sEH with an IC_{50} value of 3.4 μM. This study marks the first time that sulfuretin (**3**) was isolated from *Passiflora edulis* as well as (+)-balanophonin (**4**), and cassigarol E (**5**) were isolated from *Passiflora* genus.

Keywords – *Passiflora edulis*, Passifloraceae, Phenolic, Stilbene, sEH

Introduction

Soluble epoxide hydrolase (sEH, EC 3.3.2.10) is the major enzyme responsible for the hydrolysis of epoxy fatty acids (EpFAs) to their corresponding vicinal diols in humans and other mammals.[1] These EpFAs include the epoxides of linoleic, arachidonic, eicosapentaenoic, and docosahexaenoic acid that are produced primarily by cytochrome P450s. These natural molecules are pleiotropic endogenous mediators with key functions in inflammation,[1] pain,[2] and blood pressure regulation.[3] Increasing the levels of endogenous EpFAs by inhibiting sEH has been shown to block and resolve inflammation,[4] reduce pain,[2] lower blood pressure, and prevent cardiovascular diseases.[5] To overcome these problems, finding new inhibitors from natural plants has been investigated. A few sEH inhibitors from natural products have been identified. The results showed that natural compounds found to inhibit sEH were diverse including biflavonoids,[6] selaginellin,[7] stilbenes,[8] anthraquinone derivatives,[9] carbazole-type alkaloids (isomahanine, bisisomahanine),[10] alkylphloroglucinol derivatives, and triterpenoids.[11] These results encourage us to continue our studies in discovery of sEH inhibitors from natural sources.

From our screening results, we found that the ethanol extract of *Passiflora edulis* Sims had appreciable inhibitory activity. *Passiflora edulis* Sims (Passifloraceae), a

*Author for correspondence
To Dao Cuong, Faculty of Pharmacy, Phenikaa University, Yen Nghia, Ha Dong, Hanoi 12116, Vietnam.
Tel: +84-971-886-989; E-mail: cuong.todao@phenikaa-uni.edu.vn

Nguyen Manh Cuong, Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam.
Tel: +84-24-3791-1812; E-mail: nmcuong_inpc@yahoo.com.vn

popular tropical fruit throughout the world, is usually used for juice production,[12] and widely cultivated in South America, Africa, and Asia. In Vietnam, *P. edulis* is popularly cultivated in Tay Nguyen, Nghe An and Son La with areas of over ten thousand hectares. *P. edulis* was found to possess biological activities including anti-inflammatory,[13] antihypertensive,[14] anti-oxidant,[15] anti-tumor,[16] anti-anxiety,[17] antifungal,[18] and found to inhibit melanogenesis and promote collagen synthesis.[19] Previous studies on chemical constituents of *P. edulis* showed the presence of triterpenoids,[20] flavonoids,[21] alkaloids,[22] carotenoids,[23] stilbenoids,[19,24] oil, and tocopherols.[25] In spite of the number of studies that have been performed,[19,24,25] there has been no investigation of chemical constituents and sEH inhibitory activity of *P. edulis* seeds cultivated in Vietnam. Therefore, this paper described the isolation and structural elucidation of these compounds as well as the evaluation of their inhibitory activity on sEH.

Experimental

General experimental procedures – ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) were measured on a Bruker Avance 500 MHz spectrometer. ESI-MS was obtained from a Varian FT-MS spectrometer and MicroQ-TOF III (Bruker Daltonics, Ettlingen Germany). Optical rotations were measured on P-2000 polarimeter (JASCO, Tokyo, Japan). Column chromatography was carried out on silica gel (Si 60 F_{254}, 40-63 mesh, Merck, St. Louis, MO, USA). All solvents were redistilled before use. Pre-coated TLC plates (Si 60 F_{254}) were used for analytical purposes. Compounds were visualized under UV radiation (254, 365 nm) and by spraying plates with 10% H_2SO_4 followed by heating with a heat gun.

Plant materials – The seeds of *Passiflora edulis* Sims were provided by Nafoods Group JSC (Nghe An Province, Vietnam) in 2016 and identified by botanist Dr. Nguyen Quoc Binh, Vietnam National Museum of Nature, VAST, Hanoi, Vietnam. A voucher specimen (C-573) was deposited in the Herbarium of the Institute of Natural Products Chemistry, VAST, Vietnam.

Extraction and isolation – The dried powdered seeds (1.0 kg) of *P. edulis* were extracted three times with *n*-hexane (3 × 4.0 L) at room temperature for 3 days, filtered, and then concentrated under decreased pressure to give *n*-hexane extract (200 g) and residue. The dried residue (700 g) was then extracted three times with ethanol (3 × 3.0 L) by sonication for 6 hours. The ethanol extract (60 g) was suspended in hot-water (0.3 L) and partitioned with dichloromethane (CH_2Cl_2, 3 × 3.0 L) and ethyl acetate (EtOAc, 3 × 3.0 L) successively. The resulting fraction was concentrated under decreased pressure to give CH_2Cl_2 (5.2 g) and EtOAc (20 g) fractions, respectively. By the guided-fractionation activity, the EtOAc soluble fraction was chromatographed on a silica gel column chromatography (CC) eluting with a gradient of $CHCl_3$–MeOH (20:1 to 0:1) to afford eight fractions (Fr. E1 to Fr. E8). Fraction E3 (820 mg) was subjected on a silica gel CC eluting with a gradient of *n*-hexane–acetone (4:1 to 0:1) to afford compounds **1** (6.2 mg) and **2** (200 mg). Fraction E4 (3.5 g) was also subjected to silica gel CC eluting with a gradient of $CHCl_3$-acetone (4:1 to 0:1) to afford six sub-fractions (E4.1 to E4.6). Compounds **3** (15.8 mg) and **4** (16.2 mg) were obtained from sub-fraction E4.3 (250 mg) by using C18-RP silica gel CC and eluting with a gradient of MeOH–H_2O (1:2 to 2:1). Fraction E5 (6.5 g) was also subjected to silica gel CC eluting with a gradient of $CHCl_3$–MeOH (5:1 to 0:1) to afford eight sub-fractions (E5.1 to E5.8). The sub-fraction E5.6 (320 mg) was further subjected to C18-RP silica gel CC, eluted with a gradient of MeOH–H_2O (1:3 to 1:1) to afford compound **5** (10.2 mg).

***Trans*-resveratrol (1)** – Ivory amorphous powder; ^{1}H-NMR (500 MHz, Methanol-d_4) δ_H (ppm): 6.47 (2H, d, J = 2.0 Hz, H-2/H-6), 6.18 (1H, t, J = 2.0 Hz, H-4), 7.36 (2H, d, J = 8.5 Hz, H-2'/H-6'), 6.78 (2H, d, J = 8.5 Hz, H-3'/H-5'), 6.97 (1H, d, J = 16.0 Hz, H-8), 6.81 (1H, d, J = 16.0 Hz, H-7); ^{13}C-NMR (125 MHz, Methanol-d_4) δ_C (ppm): 159.6 (C-3/C-5), 158.3 (C-4), 141.3 (C-1), 130.4 (C-1'), 129.3 (C-8), 128.7 (C-2'/C-6'), 127.0 (C-7), 116.4 (C-3'/C-5'), 105.7 (C-2/C-6), 102.6 (C-4); ESI-MS *m/z* 229.09 $[M+H]^+$ (Calcd. for $C_{14}H_{12}O_3$).

***Trans*-piceatannol (2)** – Ivory amorphous powder; ^{1}H-NMR (500 MHz, Acetone-d_6) δ_H (ppm): 8.36 (2H, s, 3/5-OH), 8.16 (1H, br s, 4'-OH), 8.06 (1H, br s, 3'-OH), 7.07 (1H, d, J = 2.0 Hz, H-2'), 6.93 (1H, d, J = 16.5 Hz, H-8), 6.89 (1H, dd, J = 8.5, 2.0 Hz, H-6'), 6.82 (1H, d, J = 8.5 Hz, H-5'), 6.81 (1H, d, J = 16.5 Hz, H-7), 6.53 (2H, d, J = 2.0 Hz, H-2/6), 6.25 (1H, s, H-4); ^{13}C-NMR (125 MHz, Acetone-d_6) δ_C (ppm): 159.5 (C-3/C-5), 146.0 (C-3'/C-4'), 140.7 (C-1), 130.5 (C-1'), 129.2 (C-8), 126.8 (C-7), 119.8 (C-6'), 116.1 (C-5'), 113.7 (C-2'), 105.5 (C-2/C-6), 102.6 (C-4); ESI-MS *m/z* 245.08 $[M+H]^+$ (Calcd. for $C_{14}H_{12}O_4$).

Sulfuretin (3) – Yellow amorphous solid; ^{1}H-NMR (500 MHz, Methanol-d_4) δ_H (ppm): 7.63 (1H, d, J = 8.5 Hz, H-5), 7.54 (1H, d, J = 2.0 Hz, H-8), 7.26 (1H, dd, J = 8.5, 2.0 Hz, H-6), 6.86 (1H, d, J = 8.0 Hz, H-5'), 6.73 (1H, dd, J = 8.0, 2.0 Hz, H-6'), 6.72 (1H, d, J = 2.0 Hz, H-2'), 6.71 (1H, s, H-2); ^{13}C-NMR (125 MHz, Methanol-d_4)

δ_C (ppm): 184.5 (C-4), 169.8 (C-7), 168.4 (C-9), 149.3 (C-4'), 147.7 (C-3), 146.7 (C-3'), 126.8 (C-5), 126.3 (C-6'), 125.5 (C-1'), 118.9 (C-2'), 116.7 (C-5'), 114.8 (C-10), 114.6 (C-6), 114.1 (C-2), 99.3 (C-8); ESI-MS *m/z:* 271.06 $[M+H]^+$ (Calcd. for $C_{15}H_{10}O_5$).

(+)-Balanophonin (**4**) – Yellow amorphous powder; $[\alpha]_D^{25}$ +16.3° (*c* 0.05, MeOH); ^{1}H-NMR (500 MHz, Methanol-d_4) δ_H: 9.60 (1H, d, *J* = 8.0 Hz, H-9'), 7.61 (1H, d, *J* = 15.5 Hz, H-7'), 7.31 (1H, s, H-6'), 7.25 (1H, s, H-2'), 6.97 (1H, s, H-2), 6.85 (1H, d, *J* = 8.0 Hz, H-5), 6.80 (1H, d, *J* = 8.0, H-6), 6.70 (1H, dd, *J* = 15.5, 8.0 Hz, H-8'), 5.63 (1H, d, *J* = 6.5 Hz, H-7), 3.92 (3H, s, 3'-OCH_3), 3.91-3.87 (2H, m, H-9), 3.84 (3H, s, 3-OCH_3), 3.59 (1H, q, *J* = 6.5 Hz, H-8); ^{13}C-NMR (125 MHz, Methanol-d_4) δ_C (ppm): 196.1 (C-9'), 156.8 (C-4'), 156.1 (C-3'), 152.9 (C-4), 149.2 (C-3), 146.0 (C-7'), 133.9 (C-1), 131.3 (C-5), 129.6 (C-1'), 127.1 (C-8'), 120.0 (C-6), 119.8 (C-6'), 116.2 (C-5), 114.3 (C-2'), 110.6 (C-2), 90.1 (C-7), 64.5 (C-9), 56.8 (3-OCH_3), 56.4 (3'-OCH_3), 54.6 (C-8); ESI-MS *m/z:* 357.14 $[M+H]^+$ (Calcd. for $C_{20}H_{20}O_6$).

Cassigarol E (**5**) – Brown amorphous powder; $[\alpha]_D^{25}$ -56.4° (*c* 0.12, MeOH); ^{1}H-NMR (500 MHz, Methanol-d_4) δ_H (ppm): 7.15 (1H, d, *J* = 2.0 Hz, H-2), 7.09 (1H, dd, *J* = 8.5, 2.0 Hz, H-6), 6.99 (1H, d, *J* = 16.0 Hz, H-7), 6.96 (1H, d, *J* = 8.5 Hz, H-5), 6.88 (1H, d, *J* = 16.0 Hz, H-8), 6.68 (1H, d, *J* = 2.0 Hz, H-2'), 6.67 (1H, d, *J* = 8.5 Hz, H-5'), 6.50 (1H, dd, *J* = 8.5, 2.0 Hz, H-6'), 6.48 (2H, overlap, H-12/H-12'), 6.19 (1H, t, *J* = 2.0 Hz, H-14'), 6.17 (1H, t, *J* = 2.0 Hz, H-10'), 6.12 (2H, d, *J* = 2.0 Hz, H-10/H-14), 4.75 (2H, d, *J* = 2.5 Hz, H-7'/H-8'); ^{13}C-NMR (125 MHz, Methanol-d_4) δ_C (ppm): 159.6 (C-11/C-11'), 159.2 (C-13/C-13'), 146.6 (C-4'), 146.1 (C-3'), 145.4 (C-4), 145.0 (C-3), 141.0 (C-9), 140.1 (C-9'), 132.6 (C-1), 129.4 (C-7), 129.0 (C-1'), 128.4 (C-8), 121.0 (C-6'), 120.7 (C-6), 118.1 (C-5), 115.9 (C-2), 115.8 (C-5'), 115.6 (C-2'), 107.4 (C-10'/C-14'), 105.9 (C-10/C-14), 103.6 (C-12'), 102.9 (C-12), 82.2 (C-8'), 81.8 (C-7'); ESI-MS *m/z:* 487.13 $[M+H]^+$ (Calcd. for $C_{28}H_{22}O_8$).

sEH Inhibitory Activity Assay – The sEH assay was performed as described previously.[8,11] Briefly, 130 μL of sEH in 25.0 mM Bis-Tris-HCl buffer (pH 7.0) and 20.0 μL of the compounds (1 – 0.06 mM concentration) diluted in methanol, were added in 96-well plate, to which 50.0 μL of 20.0 μM PHOME was added in the mixture. After initiating the enzyme reaction at 37 °C, the products by hydrolysis of the substrate were monitored at excitation and emission of 330 and 465 nm for one hour.

Inhibitory activity (%) = $100 - [(C_{40} - C_0) - (S_{40} - S_0) / (C_{40} - C_0)] \times 100$

where C_{40} and S_{40} were the fluorescence of the control and inhibitor, respectively, after 40 min, S_0 and C_0 is the fluorescence of inhibitor and control, respectively, at 0 min. 12-(3-adamantan-1-yl-ureido)dodecanoic acid (AUDA) was used as a positive control.

Statistical Analysis – sEH inhibitory activity assay was performed in triplicate. The results are presented as the means ± standard error of the mean.

Result and Discussion

After removing the oil (vegetable oil) from the seeds of *P. edulis* by *n*-hexane, the residue was extracted with ethanol to obtain ethanol extract. In the search for sEH inhibitors from natural sources, we found that the ethanol extract of the seeds of *P. edulis* inhibited 64.7% of sEH activity at a concentration of 37.5 μg/mL. This extract was then partitioned with dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc) fractions and aqueous residue. In the preliminary experiment, we tested the inhibitory activity of these fractions at 37.5, 75.0 and 150.0 μg/mL.

Based on the results in Table 1, CH_2Cl_2- soluble fraction showed 91.8% inhibition at the concentration of 37.5 μg/mL, which was approximately 2.0-fold more potent than aqueous layer (48.7%). Interestingly, the EtOAc-soluble fraction exhibited very potently with > 100% sEH activity at the same concentration. Considering that EtOAc-soluble fraction showed the strongest action, our subsequent studies focused on the isolation of active components. This sub-fraction was subjected to column chromatography on a silica gel and C18-RP silica gel column to obtain five compounds (**1** – **5**) (Fig. 1).

Compound **1** was obtained as an ivory amorphous powder. The ^{1}H-NMR of **1** showed signals the presence of 1,3,5-trisubstituted benzene ring characterized with AB_2 system [δ_H 6.47 (2H, d, *J* = 2.0 Hz, H-2/H-6) and 6.18 (1H, t, *J* = 2.0 Hz, H-4)], and a 1,4-disubstituted benzene ring characterized with an A_2B_2 system [δ_H 7.36 (2H, d, *J* = 8.5 Hz, H-2'/H-6') and 6.78 (2H, d, *J* = 8.5 Hz, H-3'/H-5') together with *trans*-olefinic protons [δ_H 6.97 (1H, d, *J* = 16.0 Hz, H-8) and 6.81 (1H, d, *J* = 16.0 Hz, H-7)] (Fig. 1). The ^{13}C-NMR spectrum of **1** exhibited 14 carbon signals including 12 aromatic carbons [δ_C 102.6 – 159.6], belonging to two benzene rings, and two olefinic carbons [δ_C 129.3 (C-7), and 128.7 (C-8)] (Fig. 1). The ESI-MS data of **1** indicated the pseudo molecular ion at *m/z* 229.09 for the $[M+H]^+$, indicating a molecular weight of 228. Its molecular formula was determined to be $C_{14}H_{12}O_3$, according to ESI-MS, ^{1}H- and ^{13}C-NMR spectroscopic data. Based on the above evidence and comparison with reported data,[26] compound **1** was identified

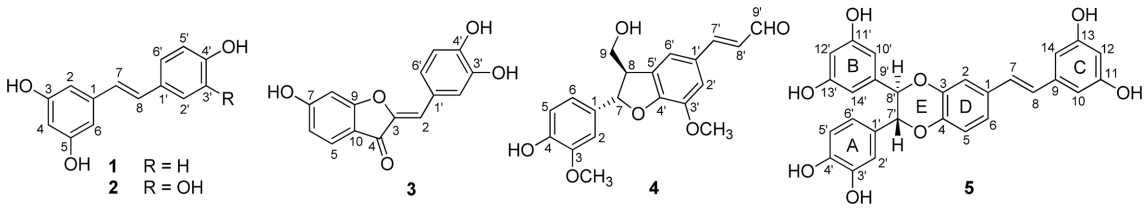

Fig. 1. The structures of isolated compounds (**1** - **5**) from the seeds of *P. edulis*.

as *trans*-resveratrol. Compound **2**, a derivative of **1**, which the ^{1}H- and ^{13}C-NMR spectra were similar to those of **1** except for the presence of 1,3,4-trisubstituted benzene ring characterized with ABX system [δ_H 7.07 (1H, d, J = 2.0 Hz, H-2'), 6.89 (1H, dd, J = 8.5, 2.0 Hz, H-6'), and 6.82 (1H, d, J = 8.5 Hz, H-5')] in **2** (Fig. 1). The ESI-MS spectrum of compound **2** showed the pseudo molecular ion at *m/z* 245.08 $[M+H]^+$, indicating the molecular formula $C_{14}H_{12}O_4$. Thus, compound **2** was identified as *trans*-piceatannol in comparison with literature data.[27] These compounds (**1** – **2**) possess the basics of stilbene skeleton and are known to inhibit anti-oxidant, anti-inflammatory, anti-diabetes and anticancer.[28]

Compound **3** was obtained as yellow amorphous solid. The ^{1}H-NMR of **3** showed signals of 1,2,4-trisubstituted benzene rings characterized with two ABX systems [δ_H 7.63 (1H, d, J = 8.5 Hz, H-5), 7.54 (1H, d, J = 2.0 Hz, H-8), and 7.26 (1H, dd, J = 8.5, 2.0 Hz, H-6); δ_H 6.86 (1H, d, J = 8.5, Hz, H-5'), 6.73 (1H, d, J = 2.0 Hz, H-2'), and 6.72 (1H, dd, J = 8.5, 2.0 Hz, H-6')], together with an olefinic proton at δ_H 6.71 (1H, s, H-2) (Fig. 1). The ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra of **3** showed signals for six aromatic quaternary carbon [δ_C 169.8 (C-7), 168.4 (C-9), 149.3 (C-4'), 146.7 (C-3'), 125.5 (C-1'), 114.8 (C-10)] and six aromatic carbons [δ_C 126.8 (C-5), 126.3 (C-6'), 118.9 (C-2'), 116.7 (C-5'), 114.6 (C-6) and 99.3 (C-8)] (Fig. 1). Furthermore, a carbonyl carbon at δ_C 184.5 (C-4), together with two olefinic carbons [δ_C 147.7 (C-3), and 114.1 (C-2)] were also observed in the ^{13}C NMR spectrum indicated that **3** was aurone skeleton (Fig. 1).[29] The ESI-MS spectrum of compound **3** showed the pseudo molecular ion at *m/z* 271.06 $[M+H]^+$, indicating the molecular formula $C_{15}H_{10}O_5$. Based on the above evidence and comparison with reported data,[30] compound **3** was identified as sulfuretin, which was isolated from *P. edulis* for the first time. This compound possessed anti-inflammatory,[31] anticancer,[32] and neuroprotective activities.[33]

Compound **4** was isolated as yellow amorphous powder. The ^{1}H-NMR spectrum of **4** showed an aldehyde proton at δ_H 9.60 (1H, d, J = 8.0 Hz, H-9'), *trans*-olefinic protons at δ_H 6.70 (1H, dd, J = 15.5, 8.0 Hz, H-8') and 7.61 (1H, d, J = 15.5 Hz, H-7'), which were assigned to a *trans*-cinnamaldehyde moiety. In addition, the ^{1}H-NMR of **4** showed two sets of aromatic protons signal [δ_H 6.97 (1H, s, H-2), 6.85 (1H, d, J = 8.0 Hz, H-5), 6.80 (1H, d, J = 8.0, H-6); δ_H 7.31 (1H, s, H-6'), 7.25 (1H, s, H-2') arising from 1,3,4-trisubstituted and 1,3,4,5-tetrasubstituted benzene rings, respectively (Fig. 1). A stereochemistry of the dihydrobenzofuran ring [δ_H 5.63 (1H, d, J = 6.5 Hz, H-7), δ_H 3.59 (1H, q, J = 6.5 Hz, H-8) and δ_H 3.91-3.87 (2H, m, H-9)], and two singlets of methoxy groups [δ_H 3.92 (3H, s, 3'-OCH_3), and 3.84 (3H, s, 3-OCH_3)] were also observed in the ^{1}H-NMR spectrum. The large proton coupling constant between H-7 and H-8 ($J_{7,8}$ = 6.5 Hz) suggested the dihydrofuran ring has a *trans*-configuration. The ^{13}C-NMR and DEPT spectra of **4** revealed the presence of twelve aromatic carbons, two olefinic carbons, an aldehyde carbon [δ_C 196.1 (C-9')], a hydroxymethyl carbon [δ_C 64.5 (C-9)] and two methoxy carbons [δ_C 56.8 (3-OCH_3), and 56.4 (3'-OCH_3)] (Fig. 1). The HMBC correlations of proton H-2 at δ_H 6.97 (1H, s) and methoxy protons at δ_H 3.84 (3H, s) to carbon signal at δ_C 149.2 (C-3), as well as proton H-2' at δ_H 7.25 (1H, s) and methoxy protons at δ_H 3.92 (3H, s) to carbon signal at δ_C 156.1 (C-3'), suggested that two methoxy groups were located at C-3 and C-3' (Fig. 1). Further analysis of these signals by the COSY, HMQC and HMBC spectra led to the partial structures of **4**. The ESI-MS data of **4** indicated the pseudo molecular ion at *m/z* 357.14 for the $[M+H]^+$, indicating a molecular weight of 356. Its molecular formula was determined to be $C_{20}H_{20}O_6$, according to ESI-MS, ^{1}H- and ^{13}C-NMR spectroscopic data. Therefore, compound **4** was identified as (+)-balanophonin,[34] which was isolated from *Passiflora* genus for the first time. This compound was known to possess anti-oxidant,[35,36] anti-cholinesterase,[36] anti-inflammatory, anticancer, and anti-neurodegenerative activities.[37]

Compound **5** was isolated as brown amorphous powder. The ^{1}H-NMR spectrum of **5** showed the presence of two ABX system signals for the A ring [δ_H 6.68 (1H, d, J = 2.0 Hz, H-2'), 6.67 (1H, d, J = 8.5 Hz, H-5'), 6.50 (1H,

dd, J = 8.5, 2.0 Hz, H-6')] and the D ring [δ_H 7.15 (1H, d, J = 2.0 Hz, H-2), 7.09 (1H, dd, J = 8.5, 2.0 Hz, H-6), 6.96 (1H, d, J = 8.5 Hz, H-5)], two sets of AB_2 system signals for the B and C rings [6.48 (2H, overlap, H-12/H-12'), 6.19 (1H, t, J = 2.0 Hz, H-14'), 6.17 (1H, t, J = 2.0 Hz, H-10'), 6.12 (2H, d, J = 2.0 Hz, H-10/H-14)], two doublets for the *trans*-olefinic protons [δ_H 6.99 (1H, d, J = 16.0 Hz, H-7), 6.88 (1H, d, J = 16.0 Hz, H-8)], and two equivalent oxybenzyl methine protons [δ_H 4.75 (2H, d, J = 2.5 Hz, H-7'/H-8')] (Fig. 1). The ^{13}C-NMR and DEPT spectra of **5** exhibited 28 carbon signals including 24 aromatic carbons [δ_C 102.9 – 159.6], belonging to four benzene rings, and two olefinic carbons [δ_C 129.4 (C-7), and 128.4 (C-8)]. In addition, the carbon signals [δ_C 82.2 (C-8') and 81.8 (C-7')] indicate the presence of two equivalent oxybenzyl methine carbons. Analysis of these signals by the COSY, HMQC and HMBC spectra led to the partial structures of compound **5** (Fig. 1). The ESI-MS data of **5** indicated the pseudo molecular ion at *m/z* 487.13 for the $[M+H]^+$, indicating a molecular weight of 486. Its molecular formula was determined to be $C_{28}H_{22}O_8$, according to ESI-MS, ^{1}H- and ^{13}C-NMR spectroscopic data. The relative configuration between the C-7' and the C-8' positions was concluded to be *cis* from the coupling constant of the two oxybenzylmethine protons (J = 2.5 Hz). Based on the above evidence and comparison with reported data,[38] compound **5** was identified as cassigarol E, which was isolated from *Passiflora* genus for the first time. Cassigarol E possessed anti-HIV-1,[39] antidiabetic,[40] and anticancer activities.[41]

The effect of isolated compounds (**1** – **5**) from *P. edulis* on sEH inhibitory activities was evaluated. The sEH inhibitory activities were determined using recombinant human sEH incubated with PHOME, an artificial substrate for fluorescence detection with AUDA (IC_{50} value 4.4 nM), a sEH inhibitor as the positive control. The result showed that compound **4** had no inhibitory (N.I) effect on the activity of enzyme sEH at a concentration of 100 μM, while compounds **1** and **2** at a concentration of 100 μM exhibited the highest sEH inhibitory activity (>100%) (Table 1). Sulfuretin (**3**), and cassigarol E (**5**) also showed a strong inhibitory effect on sEH with their inhibition values in the range of 74.6 and 77.7%, respectively (Table 1). Compounds **1** - **3**, and **5** showed inhibitory rates over 50% and were further evaluated at concentrations ranging from 6.2 to 100 μM, to elucidate the IC_{50} values. These inhibitors (**1** - **3**, and **5**) showed dose-dependent inhibition, with IC_{50} values of 14.2 ± 0.6, 3.4 ± 4.8, 15.8 ± 1.0, and 14.4 ± 0.8 μM, respectively (Table 2).

Previously, natural plant components with stilbene skeletons from other plants have also shown inhibitory effects on sEH activity. Rhapontigenin, isorhapontin and astringin from *Rheum undulatum* (Polygonaceae),[8] and 2-isopropyl-5-[(*E*)-2- phenylvinyl]benzene-1,3-diol have displayed potent sEH inhibitory activity.[42] Several stilbene from *Polygonum multiflorum* (Polygonaceae), such as (*E*)-2,3,5,4'-tetrahydroxystilbene-2-*O*-*β*-D-glucoside, (*E*)-2,3,5,4'-tetrahydroxystilbene-2-*O*-*β*-D-xyloside, and (*E*)-2,3,5,4'-tetrahydroxystilbene-2-*O*-*β*-D-(6"-*O*-acetyl)-glucoside completely inhibited sEH in a dose-dependent manner, with low IC_{50} values.[43] As in this study, some isolated stilbenes from *P. edulis* can manifest sEH inhibitory activity. Such compounds have been purified from natural medicinal plants for many years all over the world. In addition to other medicinal properties, such as anti-inflammatory, anticancer, and antioxidant activities, the sEH inhibitory activity is worthy of notice, because low-molecular-weight materials can easily reach the site of action following oral administration since they cross the blood-brain barrier.[44]

Table 1. Effects of extracts of *P. edulis* on sEH inhibitory activity

Fractions	Inhibition rate (%)[a]		
	37.5 μg/mL	75 μg/mL	150 μg/mL
EtOH	64.7 ± 0.4	97.9 ± 0.7	> 100
CH_2Cl_2	91.8 ± 3.3	95.4 ± 0.4	> 100
EtOAc	> 100	> 100	> 100
Aqueous layer	48.7 ± 0.9	61.8 ± 2.2	71.5 ± 1.5

[a] Extracts and fractions were tested three times.

Table 2. The sEH inhibitory activities of isolated compounds from *P. edulis*

Compound	100 μM (%)	IC_{50} value (μM)[a]
1	>100	14.2 ± 0.6
2	>100	3.4 ± 4.8
3	74.6 ± 0.4	15.8 ± 1.0
4	N.I	N.T
5	77.7 ± 1.3	14.4 ± 0.8
AUDA[b]	68.9 ± 0.3	4.4 ± 0.1 (nM)

N.T: Not tested.
N.I: Not inhibition.
[a] Compounds were tested three times.
[b] AUDA was used as a positive control.

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References

(1) Morisseau, C.; Hammock, B. D. *Annu. Rev. Pharmacol. Toxicol.* **2013**, *53*, 37-58.

(2) Morisseau, C.; Inceoglu, B.; Schmelzer, K.; Tsai, H. J.; Jinks, S. L.; Hegedus, C. M.; Hammock, B. D. *J. Lipid Res.* **2010**, *51*, 3481-3490.

(3) Lin, W. K.; Falck, J. R.; Wong, P. Y. *Biochem. Biophys. Res. Commun.* **1990**, *167*, 977-981.

(4) Inceoglu, B.; Jinks, S. L.; Schmelzer, K. R.; Waite, T.; Kim, I. H.; Hammock, B. D. *Life Sci*. **2006**, *79*, 2311-2319.

(5) Yu, Z.; Xu, F.; Huse, L. M.; Morisseau, C.; Draper, A. J.; Newman, J. W.; Parker, C.; Graham, L.; Engler, M. M.; Hammock, B. D.; Zeldin, D. C.; Kroetz, D. L. *Circ. Res.* **2000**, *87*, 992-998.

(6) Kim, J. H.; Tai, B. H.; Yang, S. Y.; Kim, J. E.; Kim, S. K.; Kim, Y. H. *Bull. Korean Chem. Soc.* **2015**, *36*, 300-304.

(7) Kim, J. H.; Cho, C. W.; Tai, B. H.; Yang, S. Y.; Choi, G. S.; Kang, J. S.; Kim, Y. H. *Molecules* **2015**, *20*, 21405-21414.

(8) Jo, A. R.; Kim, J. H.; Yan, X. T.; Yang, S. Y.; Kim, Y. H. *J. Enzyme Inhib. Med. Chem.* **2016**, *31*, 70-78.

(9) Lee, G. Y.; Kim, J. H.; Choi, S. K.; Kim, Y. H. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 5097-5101.

(10) Kim, J. H.; Morgan, A. M. A.; Tai, B. H.; Van, D. T.; Cuong, N. M.; Kim, Y. H. *J. Enzyme Inhib. Med. Chem.* **2016**, *31*, 640-644.

(11) Khanh, P. N.; Duc, H. V.; Huong, T. T.; Son, N. T.; Ha, V. T.; Van, D. T.; Tai, B. H.; Kim, J. E.; Jo, A. R.; Kim, Y. H.; Cuong, N. M. *Fitoterapia* **2016**, *109*, 39-44.

(12) Thokchom, R.; Mandal, G. *J. Agric. Eng. Food Technol.* **2017**, *4*, 27-30.

(13) Beninca, J. P.; Montanher, A. B.; Zucolotto, S. M.; Schenkel, E. P.; Frode, T. S. *Food Chem.* **2007**, *104*, 1097-1105.

(14) Ichimura, T.; Yamanaka, A.; Ichiba, T.; Toyokawa, T.; Kamada, Y.; Tamamura, T.; Maruyama, S. *Biosci. Biotechnol. Biochem.* **2006**, *70,* 718-721.

(15) Lourith, N.; Kanlayavattanakul, M. *J. Oleo Sci.* **2013**, *64*, 235-240.

(16) Puricelli, L.; Dell'Aica, I.; Sartorb, L.; Garbisa, S.; Caniato, R. *Fitoterapia* **2003**, *74,* 302-304.

(17) Coleta, M.; Campos, M. G.; Cotrim, M. D.; Proenca da Cunha, A. *Pharmacopsychiatry* **2001**, *34*, S20-S21.

(18) Pelegrini, P. B.; Noronha, E. F.; Muniz, M. A.; Vasconcelos, I. M.; Chiarello, M. D.; Oliveira, J. T.; Franco, O. L. *Biochim. Biophys. Acta.* **2006***, 1764*, 1141-1146.

(19) Matsui, Y.; Sugiyama, K.; Kamei, M.; Takahashi, T.; Suzuki, T.; Katagata, Y.; Ito, T. *J. Agric. Food Chem.* **2010**, *58*, 11112-11118.

(20) Bombardelli, E.; Bonati, A.; Gabetta, B.; Martinelli, E. M.; Mustich, G.; Danieli, G. *Phytochemisty* **1975**, *14,* 2661-2665.

(21) Mareck, U.; Herrmann, K.; Galensa, R.; Wray, V. *Phytochemistry* **1991**, *30*, 3486-3487.

(22) Seigler, D. S.; Pauli, G. F.; Nahrstedt, A.; Leen, R. *Phytochemistry* **2002**, *60,* 873-882.

(23) Pereira, C. A.; Yariwake, J. H.; Lancas, F. M.; Wauters, J. N.; Tits, M.; Angenot, L. *Phytochem. Anal.* **2004**, *15,* 241-248.

(24) Sano, S.; Sugiyama, K.; Ito, T.; Katano, Y.; Ishihata, A. *J. Agric. Food Chem.* **2011**, *59*, 6209-6213.

(25) Piombo, G.; Barouh N.; Barea, B.; Boulanger, R.; Brat, P.; Pina, M.; Villeneuve, P. *OCL* **2006**, *13*, 195-199.

(26) Mattivi, F.; Reniero, F.; Korhammer, S. *J. Agric. Food Chem.* **1995**, *43*, 1820-1823.

(27) Mathi, P.; Das, S.; Nikhil, K.; Roy, P.; Yerra, S.; Ravada, S. R.; Bokka, V. R.; Botlagunta, M. *Int. J. Prev. Med.* **2015**, *6*, 101.

(28) Kukreja, A.; Wadhwa, N.; Tiwari, A. *J. Blood Disord. Transfus.* **2014**, *5*, 240.

(29) Gaur, R.; Kumar, S.; Trivedi, P.; Bhakuni, R. S.; Bawankule, D. U.; Pal, A.; Shanker, K. *Nat. Prod. Commun.* **2010**, *5*, 1243-1246.

(30) Júnior, G. M. V.; Sousa, C. M. M.; Cavalheiro, A. J.; Lago, J. H. G.; Chaves, M. H. *Helv. Chim. Acta* **2008**, *91*, 2159-2167.

(31) Lee, D. S.; Jeong, G. S.; Li, B.; Park, H.; Kim, Y. C. *Int. Immunopharmacol*. **2010**, *10*, 850-858.

(32) Lee, K. W.; Chung, K. S.; Seo, J. H.; Yim, S. V.; Park, H. J.; Choi, J. H.; Lee, K. T. *J. Cell. Biochem*. **2012**, *113*, 2835-2844.

(33) Pariyar, R.; Lamichhane, R.; Jung, H. J.; Kim, S. Y.; Seo, J. *Int. J. Mol. Sci*. **2017**, *18*, E2753.

(34) Li, J. L.; Li, N.; Xing, S. S.; Zhang, N.; Li, B. B.; Chen, J. G.; Ahn, J. S.; Cui, L. *Arch. Pharm. Res.* **2017**, *40*, 1265-1270.

(35) Esposito, T.; Sansone, F.; Franceschelli, S.; Del Gaudio, P.; Picerno, P.; Aquino, R. P.; Mencherini, T. *Int. J. Mol. Sci.* **2017**, *18*, E392.

(36) Boğa, M.; Yilmaz, P. K.; Cebe, D. B.; Fatima, M.; Siddiqui, B. S.; Kolak, U. *Z. Naturforsch C J. Biosci.* **2014**, *69*, 381-390.

(37) Lim, S. Y.; Subedi, L.; Shin, D.; Kim, C. S.; Lee, K. R.; Kim, S. Y. *Biomol. Ther.* **2017**, *25*, 519-527.

(38) Baba, K.; Kido, T.; Taniguchi, M.; Kozawaqa, M. *Phytochemistry* **1994**, *36*, 1509-1513.

(39) Bunluepuech, K.; Wattanapiromsakul, C.; Tewtrakul, S. *Songklanakarin J. Sci. Technol.* **2013**, *35*, 665-669.

(40) Tran, H. H. T.; Nguyen, M. C.; Le, H. T.; Nguyen, T. L.; Pham, T. B.; Chau, V. M.; Nguyen, H. N.; Nguyen, T. D. *Pharm. Biol.* **2014**, *52*, 74-77.

(41) Yuenyongsawad, S.; Bunluepuech, K.; Wattanapiromsakul, C.; Tewtrakul, S. *Songklanakarin J. Sci. Technol.* **2014**, *36*, 189-194.

(42) Buscató, E.; Büttner, D.; Brüggerhoff, A.; Klingler, F. M.; Weber, J.; Scholz, B.; Zivković, A.; Marschalek, R.; Stark, H.; Steinhilber, D.; Bode, H. B.; Proschak, E. *ChemMedChem* **2013**, *8*, 919-923.

(43) Sun, Y. N.; Li, W.; Kim, J. H.; Yan, X. T.; Kim, J. E.; Yang, S. Y.; Kim, Y. H. *Arch. Pharm. Res.* **2015**, *38*, 998-1004.

(44) Broadwell, R. D.; Sofroniew, M. V. *Exp. Neurol.* **1993**, *120*, 245-263.

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