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Christopher R. McCurdy, PhD, FAAPS is a broadly trained medicinal chemist, behavioral pharmacologist and pharmacist whose research focuses on the design, synthesis and development of drugs to treat pain and drug abuse. For over 20 years, much of his research has focused on opioid, Neuropeptide FF, and sigma receptor ligand/probe design, synthesis, pharmacological evaluation and development. He has been successful in discovering unique and selective tools for sigma receptors, NPFF receptors and opioid receptors. He is an internationally recognized expert on Kratom (Mitragyna speciosa), that is under investigation for opioid withdrawal syndrome. A significant portion of his career has been dedicated to the development of novel sigma receptor ligands, in collaboration with a variety of interdisciplinary groups, to generate and optimize selective ligands which could serve as critical experimental tools, and more recently, as potential medication development leads to attenuate the effects of cocaine, methamphetamine and pain. Most notably, he has developed a PET/MR imaging diagnostic agent for visualizing the origins of chronic, neuropathic pain by interacting with sigma receptors at the site of nerve damage. Human Phase 1 clinical trials are currently underway at Stanford University. In addition to his discovery chemistry roles, McCurdy serves as the director of the UF Translational Drug Development Core. He is a member of the Editorial Advisory Board for the Journal of Medicinal Chemistry and is a Senior Editor for Medicinal Chemistry Research. Dr. McCurdy is servings as the 2017-2018 President of the American Association of Pharmaceutical Scientists (AAPS) and also serves as the Co-Chair of the Special Interest Group on Drug Design and Discovery (DDD) of the International Pharmaceutical Federation (FIP). He has published over 100 peer-reviewed manuscripts and has been issued 4 patents.

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Dr. Paulo Carvalho was a compounding pharmacist in Brazil, where he also earned his Ph.D. in Medicinal Chemistry from Universidade de São Paulo. Dr. Carvalho's research interests include natural products chemistry, synthesis of drugs against tropical diseases and HPLC-MS analysis of drugs and their metabolites in biological fluids. He is currently an Associate Professor at the Feik School of Pharmacy, University of the Incarnate Word, San Antonio, TX.

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Dr. Kim received his baccalaureate in Biology and doctoral degree in Medicinal Chemistry at the University of Mississippi. His doctoral research projects were involved medicinal/organic chemistry with study on structural-activity relationship on bio-active natural products. Currently, Dr. Kim is an Assistant Professor of Chemistry, Bluefield State College, Bluefield, WV. Away from his teaching and researching, he likes to travel and is a big Ole Miss Sports fan.

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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Chemical constituents of *Piper aduncum* and their inhibitory effects on soluble epoxide hydrolase and tyrosinase

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Abstract A new compound, 2(*S*)-isobutanol 2-O-β-D-arabinopyranosyl(1→6)-O-β-D-glucopyranoside (**1**), along with ten known compounds (**2–11**) were isolated from *Piper aduncum* L. leaves. The effects of these compounds on soluble epoxide hydrolase and tyrosinase inhibition were evaluated. Among them, compounds **3**, **8**, and **9** exhibited significant tyrosinase inhibitory activity with IC_{50} values of 39.3 ± 1.8, 41.3 ± 2.2, and 37.5 ± 2.7 μM, respectively. However, the effects of isolated compounds on soluble epoxide hydrolase inhibition were weak or absent, and compounds **4** and **11** showed the highest inhibitory activity with values of 61.2 ± 4.3 and 60.6 ± 3.7 % at a concentration of 100 μM.

Keywords *Piper aduncum* · Alkyl glycoside · Soluble epoxide hydrolase · Tyrosinase inhibition

Abbreviations

AUDA	12-(3-Adamantan-1-yl-ureido)-dodecanoic acid
DHETs	Dihydroxyeicosatrienoic acids
EETs	Epoxyeicosatrienoic acids
PHOME	3-Phenyl-cyano(6-methoxy-2-naphthalenyl) methyl ester-2-oxiraneacetic acid
sEH	Soluble epoxide hydrolase

Introduction

Epoxide hydrolases are a family of enzymes that catalyze the hydrolysis of epoxides or arene oxides to their corresponding diols by the addition of water. The epoxide hydrolase family consists of five main subtypes: soluble epoxide hydrolase (sEH), microsomal epoxide hydrolase, leukotriene A_4 hydrolase, hepoxilin A_3 hydrolase, and cholesterol 5,6-oxide hydrolase (Fretland and Omiecinski 2000). sEH has been described as a key enzyme in the metabolism of eicosanoid epoxides and plays crucial roles in the metabolism of epoxyeicosatrienoic acids (EETs), leukotoxins (LTX) to their less active metabolites, dihydroxyeicosatrienoic acids, and leukotoxin diol (Spector and Norris 2007). EETs and sEH inhibitors have been extensively characterized in a variety of cellular assays and disease models. Both EETs and sEH inhibitors exhibit activities including the modulation of potassium channels, antiapoptotic effects in endothelial cells, anti-inflammatory properties, and inhibition of the nuclear factor kappa B signaling pathway.

Electronic supplementary material The online version of this article (doi:10.1007/s00044-016-1735-3) contains supplementary material, which is available to authorized users.

Bui Thi Thuy Luyen and Nguyen Phuong Thao have contributed equally to this work.

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Tyrosinase, a key enzyme in melanin biosynthesis, is a multifunctional, glycosylated, and copper-containing oxidase. Tyrosinase catalyzes three different reactions in the biosynthetic pathway of melanin in melanocytes: the hydroxylation of tyrosine to L-DOPA and oxidation of L-DOPA to dopaquinone; furthermore, in humans, dopaquinone is converted by a series of complex reactions to melanin. It is also responsible for enzymatic browning reactions in damaged fruits and vegetables during post-harvest handling and processing, which makes the identification of tyrosinase inhibitors extremely important. Therefore, tyrosinase inhibitors have received considerable attention in numerous previous studies. The development of tyrosinase inhibitors has become important in many products such as foods, cosmetics, and medicines that may be useful for the prevention or treatment of pigmentation disorders. Some tyrosinase inhibitors have been identified previously, including kojic acid, arbutin, catechins, hydroquinone, and azelaic acid. However, plants are a rich source of tyrosinase inhibitors containing polyphenols, benzaldehyde and benzoate derivatives, lipids, and steroids (Chang 2009).

The genus *Piper* (Piperaceae) is known for producing a large number of physiologically active compounds and is widely used in folklore medicine in the West Indies and Latin America. The Piperaceae family, to which the genus *Piper* belongs, comprises approximately 2000 species distributed in the tropical regions of the world. *Piper aduncum* L. is widely used in folk medicine, to treat stomach aches, and as an anti-inflammatory and antiseptic to heal wounds (Morandim et al. 2009). Previous studies have reported that *P. aduncum* has antihypertensive, gastroprotective, hepatoprotective, anti-tumorigenic, hypolipidemic, anti-inflammatory, antioxidant, anti-genotoxic, and anti-breast cancer effects in rats (Arroyo-Acevedo et al. 2015). Phytochemical studies of *P. aduncum* reported the isolation of chalcones, flavanones, dihydrochalcones, phenylpropanoids, chromene, and benzoic acid derivatives (Baldoqui et al. 1999; Morandim et al. 2005; Orjala et al. 1994).

In a continuing study on bioactive compounds from natural sources (Thao et al. 2016a, b), we report the isolation and structural elucidation of a new alkyl glycoside (**1**) and ten known compounds (**2–11**) from *P. aduncum* leaves (Fig. 1). Additionally, the sEH and tyrosinase inhibiting effects of these compounds were evaluated.

Results and discussion

Phytochemical analysis

Compound **1** was obtained as a colorless oil. High-resolution electrospray ionization mass spectra (HR-ESI-MS) showed a pseudo-molecular ion peak at *m/z* 391.1582 $[M+Na]^+$, which revealed its molecular formula to be $C_{15}H_{28}O_{10}$. The ^1H nuclear magnetic resonance (NMR) spectra of **1** showed signals of two methyl protons, which appeared as a doublet H-1 [δ_H 1.26 (d, $J = 6.0$ Hz)] and a

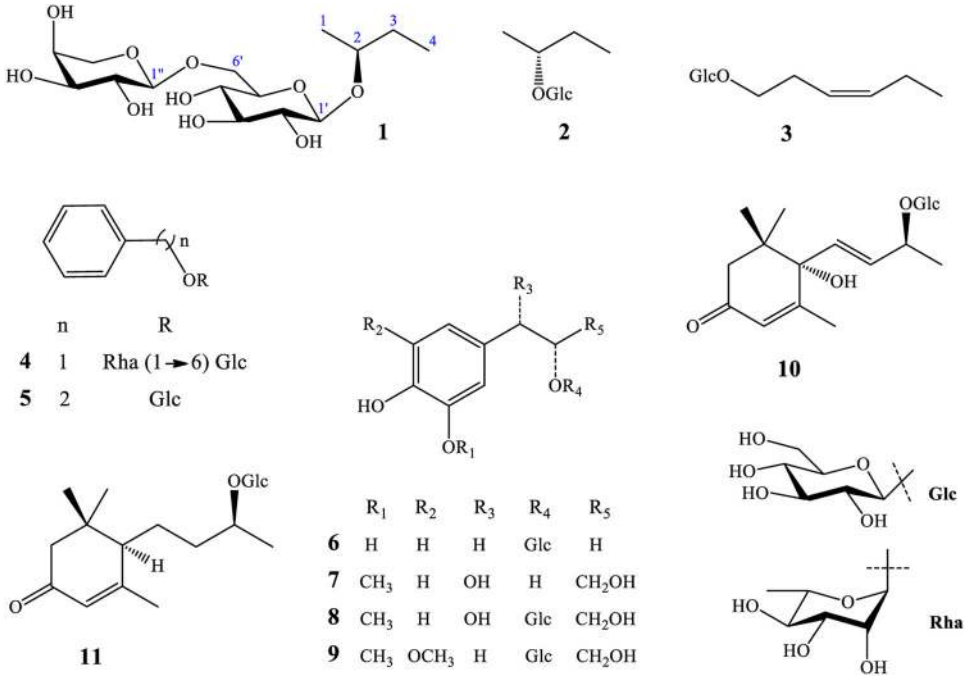

Fig. 1 Chemical structures of compounds **1–11** isolated from *P. aduncum* leaves

triplet H-4 [δ_H 0.98 (t, $J = 9.0$ Hz)], an oxygenated methine proton H-2 (δ_H 3.89, m) and methylene proton signals [δ_H 1.53 through 1.65]. The signals of two anomeric protons, H-1′ (δ_H 4.36, d, $J = 7.5$) and H-1″ (δ_H 4.37, d, $J = 6.5$), indicated the presence of two sugar units (Table 1). The anomeric proton signal of H-1′ was attributed to a β-glucosyl unit from the large coupling constant ($^3J_{1,2} = 7.5$ Hz) (Thao et al. 2016a).

The ^{13}C NMR spectra of **1** displayed four carbon signals of aglycone, including two methyls (δ_C 9.9 and 21.5), one methylene (δ_C 30.1), and an oxygenated methine carbon (δ_C 79.7), together with 11 carbon resonant signals of the sugar moiety (δ_C 65.6 through 105.1). Three individual correlation spectroscopy (COSY) correlation spin systems of H-1/H-2/H-3/H-4, H-1′/H-2′/H-3′/H-4′/H-5′/H-6′, and H-1″/H-2″/H-3″/H-4″/H-5″ were observed in the COSY spectra of **1**, supporting the backbone proton assignments of aglycone and two sugar units. The Heteronuclear Multiple Bond Correlation (HMBC) correlations of arabinose H-1″ (δ_H 4.37)/glucose C-6′ (δ_C 69.4) and glucose H_2-6′ (δ_H 4.12 and 3.87)/arabinose C-1″ (δ_C 105.1) were indicative of an arabinosyl attached at C-6′ of glucose. Additionally, the position of the sugar moiety in **1** was identified at C-2 based on the HMBC correlations of anomeric proton H-1′ (δ_H 4.36)/C-2 (δ_C 79.7) and H-2 (δ_H 3.89)/C-1′ (δ_C 103.8). Additionally, the presence of D-glucose and D-arabinose in **1** were determined based on acid hydrolysis, TLC, GC analysis, and comparison with authentic D-glucose and D-arabinose ("Materials and methods" section).

The relative configuration at C-2 of **1** was suggested to be identical with that of 2(*S*)- and 2(*R*)-butanol-2-O-β-D-galactopyranoside (Crout et al. 1990) on the basis of the similarity of the sign of NMR data and optical rotations ($[\alpha]_D$). Agreement of the NMR chemical shift at C-2 (δ_H 3.89/δ_C 79.7) and $[\alpha]_D^{24}$: −56.4 (*c* 0.20, MeOH) of **1** with those of 2(*S*)- and 2(*R*)-butanol-2-O-β-D-galactopyranoside [δ_H 4.40/δ_C 79.4 and $[\alpha]_D^{25}$: −79.1 (*c* 0.30, MeOH) for 2(*S*) and δ_H 4.40/δ_C 78.6 and $[\alpha]_D^{25}$: +22.0 (*c* 0.25, MeOH) for 2(*R*)] was suggestive of an '*S*' configuration at C-2 (Crout et al. 1990; Sigurskiold et al. 1992). Thus, compound **1** was identified as 2(*S*) isobutanol 2-O-β-D-arabinopyranosyl (1→6)-O-β-D-glucopyranoside.

Other compounds were identified by comparison of their NMR data to previous reports, including (2*R*)-butyl-O-β-D-glucopyranoside (**2**) (Crout et al. 1990), (*Z*)-3-hexenyl-O-β-D-glucopyranoside (**3**) (Mizutani et al. 1988), 1′-O-benzyl-α-L-rhamnopyranosyl-(1″→6′)-β-D-glucopyranoside (**4**) (Kawahara et al. 2005), 2-phenylethyl-O-β-D-glucopyranoside (**5**) (Umehara et al. 1988), 2-(3,4-dihydroxy)phenylethyl-O-β-D-glucopyranoside (**6**) (Franzyk et al. 2004), guaiacylglycerol (**7**) (Okuyama et al. 1998), guaiacylglycerol 8-O-β-D-glucopyranoside (**8**) (Ishimaru et al. 1987), xylocoside B [1-hydroxy-3-(4-hydroxy-3,5-dimethoxyphenyl)prop-2-yl β-glucopyranoside] (**9**) (Xu et al. 2008), (6*S*,9*S*)-roseoside (**10**) (Yamano and Ito 2005), and (6*R*,9*S*)-9-hydroxymegastigman-4-en-3-one 9-O-β-D-glucopyranosides (**11**) (Matsunami et al. 2010).

Table 1 1H and ^{13}C NMR spectroscopic data (CD_3OD) for compound **1**

Pos.	δ_H[a] (mult., *J* in Hz)	δ_C[b]
1	1.26 (d, 6.0)	21.5, CH_3
2	3.89[c]	79.7, CH
3	1.53 (m)	30.1, CH_2
	1.65 (m)	
4	0.98 (t, 9.0)	9.9, CH_3
Glucose (inner)		
1′	4.36 (d, 7.5)	103.8, CH
2′	3.19 (dd, 7.5, 8.5)	75.2, CH
3′	3.64[c]	77.9, CH
4′	3.36 (t, 9.0)	72.3, CH
5′	3.60 (m)	76.5, CH
6′	4.12 (dd, 3.0, 12.0)	69.4, CH_2
	3.87 (dd, 4.5, 12.0)	
Arabinose (terminal)		
1″	4.37 (d, 6.5)	105.1, CH
2″	3.23[c]	71.5, CH
3″	3.56 (t, 3.0, 9.0)	74.1, CH
4″	3.46 (m)	69.3, CH
5″	3.92 (dd, 2.0, 12.0)	65.6, CH_2
	3.22 (dd, 3.5, 12.0)	

Assignments were done by HMQC, HMBC, and COSY

[a] 600 MHz

[b] 150 MHz

[c] Overlapped signals

Biological activity

The sEH inhibitory activity of isolated compounds was then evaluated using a fluorescent method based on hydrolysis of the specific substrate PHOME in the presence of sEH enzyme. AUDA (150 nM), one of the most effective sEH inhibitors, was used as a positive control. At a concentration of 100 μM, compounds **4** and **11** showed the highest inhibitory activity of 61.2 ± 4.3 and 60.6 ± 3.7 %, respectively (Table 2). Other compounds inhibited weakly or had no activity.

All compounds were investigated for their tyrosinase inhibition activity. At concentrations of 50.0 and 100 μM, compounds **2**, **6**, **10**, and **11** showed moderate activity, with tyrosinase inhibitory activities ranging from 14.4 to 46.1 % (Fig. 2). Compounds **1**, **3–5**, and **7–9** showed significant tyrosinase enzyme inhibitory activity, with inhibitory activities

ranging from 38.5 to 87.3 % and were assessed for further investigation. The effects of those compounds were examined at various concentrations, ranging from 6.25 to 100 μM, and the 50 % IC_{50} was calculated using a dose-dependent response curve (Table 3). Kojic acid was used as a positive control with an IC_{50} value of 12.9 ± 4.1 μM. The results showed that compounds **3**, **8**, and **9** exhibited significant tyrosinase inhibitory activity with IC_{50} values of 39.3 ± 1.8, 41.3 ± 2.2, and 37.5 ± 2.7 μM, respectively. Compounds **1**, **4**, **5**, and **7** showed moderate inhibitory activity, with IC_{50} values of 76.3 ± 4.1, 80.1 ± 3.5, 63.4 ± 2.6, and 51.1 ± 1.7 μM, respectively.

Materials and methods

General experimental procedures

Optical rotation was recorded on a JASCO DIP-370 automatic digital polarimeter. The UV spectrum was recorded on a JASCO V-630 spectrophotometer. IR spectra were obtained on a Bruker TENSOR 37 FT-IR spectrometer. The NMR spectra were measured using a JEOL ECA 600 spectrometer (JEOL, Tokyo, Japan) with Tetramethylsilane as the internal standard. The electrospray ionization mass spectra were performed on an AGILENT 1100 LC-MSD trap spectrometer (Agilent Technologies, Palo Alto, CA, USA). The HR-ESI-MS were obtained from an Agilent 6530 Accurate-Mass Q-TOF LC/MS system. Gas chromatography (GC) spectra were recorded on a Shidmazu-2010 spectrometer (Shimadzu, Kyoto, Japan). Silica gel (70–230, 230–400 mesh, Merck, Whitehouse Station, NJ), YMC RP-18 resins (75 μm, Fuji Silysia Chemical Ltd., Kasugai, Japan) were used as absorbents in the column chromatography (CC). Thin layer chromatography (TLC) plates (silica gel 60 F_{254} and RP-18 F_{254}, 0.25 μm, Merck) were purchased from Merck KGaA (Darmstadt, Germany). Spots were detected under UV radiation (254 and 365 nm) and by spraying the plates with 10 % H_2SO_4 followed by heating with a heat gun. Other chemical reagents and standard compounds were purchased from Sigma-Aldrich (St. Louis, MO).

Plant material

The leaves of *Piper aduncum* L. were collected from Coblong-Bandung, West Java, Indonesia in September 2014 and taxonomically identified by the staff at the Herbarium Laboratory, Department of Biology, School of Life Sciences and Technology, Bandung Institute of Technology, Bandung, West Java, Indonesia. A voucher specimen

Table 2 Effects of compounds **1–11** (100 μM) on sEH inhibitory activity determined using the fluorometric method

Compounds	Inhibition (%)
1	5.9 ± 2.2
2	9.5 ± 3.6
3	1.3 ± 2.2
4	61.2 ± 4.3
5	5.7 ± 1.8
6	15.5 ± 1.5
7	20.3 ± 2.4
8	30.5 ± 2.2
9	18.0 ± 3.1
10	0.5 ± 1.0
11	60.6 ± 3.7
AUDA	89.8 ± 1.7

AUDA (150 nM) was used as a positive sEH inhibitor

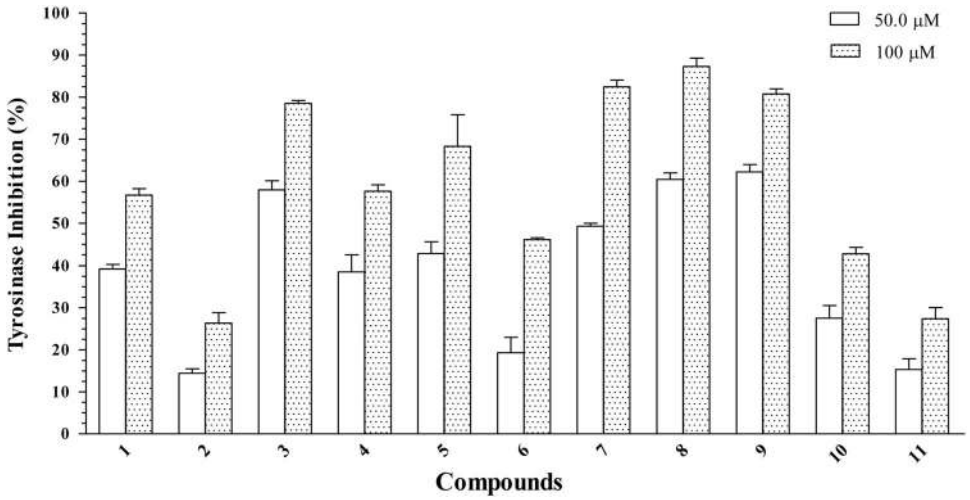

Fig. 2 Tyrosinase inhibition effects of compounds isolated from *P. aduncum* leaves. The data were presented as inhibition rate (%). Data represent the mean ± SD of at least three independent experiments performed in triplicates

Table 3 Tyrosinase inhibition effects of selected compounds isolated from *P. aduncum*

Compounds	IC_{50} values (μM)
1	76.3 ± 4.1
3	39.3 ± 1.8
4	80.1 ± 3.5
5	63.4 ± 2.6
7	51.1 ± 1.7
8	41.3 ± 2.2
9	37.5 ± 2.7
Kojic acid	12.9 ± 4.1

Kojic acid was used as a positive control

(BIT-1481) was deposited at the Herbarium of the Department of Biology, School of Life Sciences and Technology, Bandung Institute of Technology.

Chemicals and reagents

PHOME, purified recombinant sEH, 14,15-EET, 14,15-DHET, and leukotoxindiol (+/−)9(10)-dihydroxy-octadec-12-enoic acid (9,10-DiHOME) were purchased from Cayman Chemical (Ann Arbor, MI). AUDA, butyl ester was purchased from Cayman Chemical (Ann Arbor, MI). 6-Methoxy-2-naphtaldehyde (internal standard for fluorometric assays) was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Bis-Tris and Greiner 96-well black plates were from Sigma-Aldrich (St. Louis, MO). The natural compounds tested in this study were isolated from Library of Natural Products, College of Pharmacy, Chungnam National University. Information about their isolation method, chemical structure, and purity is provided in the references listed in main text.

Extraction and isolation

The dried leaves of *P. aduncum* L. (3.2 kg) were extracted with 70 % methanol (30 L × 3 times) under reflux condition. Evaporation of the solvent under reduced pressure gave MeOH extract (379 g). The crude extract was suspended in H_2O and successively separated with CH_2Cl_2 and EtOAc to yield CH_2Cl_2 (93 g), EtOAc (27 g) fractions, and water layer.

The EtOAc fraction (27 g) was fractionated on a silica gel CC eluting with gradient solvent systems of CH_2Cl_2–MeOH (0–100 % MeOH, step-wise) to obtain six fractions (C.1–C.6). Compounds **3** (40 mg) and **5** (38 mg) were isolated from fraction C.3 by YMC reverse-phase (RP)-18 CC using MeOH–H_2O (1/1) as eluent and further purified by other YMC RP-18 CC eluting with acetone-H_2O (1/4).

The water layer was fractionated on a Diaion® HP-20 CC eluting with gradient solvent systems of MeOH–H_2O (0–100 % MeOH, step-wise) to obtain four fractions [D (50 g), E (16 g), F (15 g), and G (30 g)]. Fraction D was separated by CC over silica gel, eluting with gradient solvent systems of CH_2Cl_2–MeOH (0–100 %, step by step) to obtain five fractions (D.1–D.5). Fraction D.2 was separated by silica gel CC using CH_2Cl_2–MeOH (3/1) and further purified by silica gel CC, using EtOAc-MeOH (15/1) as eluents to afford **7** (20 mg). Fraction D.4 was separated on silica gel CC eluting with EtOAc-MeOH-H_2O (3/1/0.1) and further purified by Sephadex™ LH-20 CC using MeOH–H_2O (1/1) as eluent to give compound **8** (10 mg). Fraction E was separated by YMC-RP18 CC using the gradient solvent system of MeOH–H_2O (1/3-1/1) to give five fractions (E.1–E.5). Fraction E.1 was further isolated by a silica gel CC eluted with EtOAc-MeOH (1/1) to obtain three sub-fractions (E.1.1–E.1.3). Fraction E.1.1 was purified by silica gel CC using CH_2Cl_2–MeOH–H_2O (4/1/0.1, etc.) as eluents to afford compounds **2** (10 mg) and **6** (40 mg). Fraction E.1.2 was separated by YMC-RP_{18} CC using MeOH–H_2O (1/3) and further purified by silica gel CC, using CH_2Cl_2–MeOH–H_2O (4/1/0.1) as eluents to afford compounds **1** (60 mg) and **9** (17 mg). Similarly, compounds **4** (150 mg) and **10** (200 mg) were obtained from the fraction E.2 by silica gel CC using EtOAc–MeOH–H_2O (4:1:0.1) as eluent. Finally, compound **11** (15 mg) was obtained from the fraction E.4 by silica gel CC using EtOAc–MeOH–H_2O (5:1:0.1) as eluent.

2(S)-Isobutanol 2-O-β-D-arabinopyranosyl(1→6)-O-β-D-glucopyranoside (**1**): Colorless oil; $[\alpha]_D^{24}$: −56.4 (*c* 0.20, MeOH); IR ν_{max} (KBr): 3390, 2965, 1451, 1076, and 1035 cm^{-1}; UV (MeOH) λ_{max} (log ε): 256 (3.89) nm; HR-ESI-MS (positive-ion mode): *m/z* 391.1582 $[M+Na]^+$ (calcd. $C_{15}H_{28}NaO_{10}$ for 391.1585); ^1H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) data are given in the Table 1.

Acid hydrolysis and sugar identification

Compound **1** (2.0 mg) was dissolved in 1.0 N HCl (dioxane-H_2O, 1:1, v/v, 1.0 mL) and then heated to 80 °C in a water bath for 3 h. The acidic solution was neutralized with silver carbonate and the solvent thoroughly driven out under N_2 gas overnight. After extraction with ethyl acetate, the aqueous layer was first subjected to TLC analysis (individual and co-analysis with standard sample: glucose (R_f 0.29), arabinose (R_f 0.50) ($CHCl_3$/MeOH/H_2O, 3:2:0.3)) and then concentrated to dryness using N_2 gas. The residue was dissolved in 0.1 mL of dry pyridine, and then L-cysteine methyl ester hydrochloride in pyridine (0.06 M, 0.1 mL) was added to the solution. The reaction mixture was heated at 60 °C for 2 h, and 0.1 mL of trimethylsilylimidazole

solution was added, followed by heating at 60 °C for 1.5 h. The dried product was partitioned with *n*-hexane and H_2O (0.1 mL, each), and the organic layer was analyzed by gas liquid chromatography (GC): Column: SPB-1 (0.25 mm × 30 m); detector FID, column temp 210 °C, injector temperature 270 °C, detector temperature 300 °C, carrier gas He. The absolute configuration of the monosaccharide was confirmed to be D-glucose and D-arabinose by comparison of the retention time of the monosaccharide derivative (t_R 14.11 and 7.14 min, respectively) with that of authentic sugar derivative samples prepared in the same manner (the retention times of standards D-glucose, D-arabinose were 14.12 and 7.14 min, respectively (Thao et al. 2016a)).

sEH inhibitory activity

sEH inhibitory activity was determined using a hydrolysis reaction of PHOME in the presence of the sEH enzyme. The final reaction volume was 200 µL, and contained 25 mM Bis-Tris buffer (including 0.1 % bovine serum albumin, pH 7.0), 1.0 µM PHOME, 3.0 nM sEH enzyme, and various concentrations of samples or the positive control AUDA (150 nM). Reaction systems were incubated at 30 °C for 1 h, and fluorescence intensity was then monitored every 3 min (during 1 h) using a Genios microplate reader (Tecan, Mannedorf, Switzerland) at excitation and emission wavelengths of 320 and 465 nm, respectively. sEH inhibitory activity for each sample was calculated as follows:

$$\text{sEH inhibitory activity } (\%) = 100 - \left(\frac{\int \text{SA}}{\int \text{CA}} \times 100\right),$$

where $\int$SA and $\int$CA are the integrated areas under the curve for the sample and control reactions, respectively. A sEH inhibitory activity value of 0 ($\int \text{SA}/\int \text{CA} = 1$) corresponds to a sample lacking both inhibition of sEH enzyme and PHOME hydrolysis. Nevertheless, a maximum theoretical sEH inhibitory activity value of 100 would indicate complete inhibition of PHOME hydrolysis throughout the assay ($\int \text{SA} = 0$).

Tyrosinase assay

Tyrosinase assay was performed as previously described (Khatib et al. 2005; Wang et al. 2014). Briefly, all samples were dissolved in DMSO at 10.0 mM and then diluted with buffer to the required concentrations. First, 50.0 µL solution of tyrosinase (113.3 units/mL) in 90.0 µL phosphate buffer (pH 6.8) was mixed with 10.0 µL of test samples in 96-well microplates. After adding 50.0 µL of 2.0 mM L-tyrosine solution, the progress of the reaction of the substrate (L-tyrosine) and the enzyme was monitored at 37 °C every 1 min for 20 min using a plate reader at 475 nm by spectrophotometric measurement of the dopachrome formation. Buffer and kojic acid were used as a vehicle and positive control, respectively. Each assay was conducted as three separate replicates. The percentage of inhibition of tyrosinase activity was calculated by the difference of absorbance between test samples and control. Inhibition activity was calculated from equation:

$$I = 100 - \frac{A_{\text{sample}}}{A_{\text{blank}}} \times 100,$$

where I is inhibition (%), A_{sample} and A_{blank} are relative absorption of sample and blank at 20 min in comparison to initial reaction 0 min.

Statistical analysis

All data represented the mean ± SD of at least three independent experiments performed in triplicates. Statistical significance was indicated as determined by one-way ANOVA followed by Dunnett's multiple comparison test, $P < 0.05$, using GraphPad Prism 6 program (GraphPad Software Inc., San Diego, CA, USA).

Conclusions

In summary, here we reported 11 phenolic compounds from *P.* aduncum. Our results indicate that the phenolic components of *P. aduncum* have significant tyrosinase inhibitory activity. However, the effects of isolated compounds on sEH inhibition were not found. This is the first study on the chemical constituents of *P. aduncum* with sEH and tyrosinase inhibitory activities.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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LEMBAR HASIL PENILAIAN
SEJAWAT SEBIDANG atau *PEER REVIEW*

KARYA ILMIAH : JURNAL ILMIAH

Judul Karya Ilmiah (Artikel) : Chemical constituents of Piper aduncum and their inhibitory effects on soluble epoxide hydrolase and tyrosinase

Jumlah Penulis : 7 Orang

Nama-nama Penulis : Bui Thi Thuy Luyen, Nguyen Phuong Thao, Wahyu Widowati, Nurul Fauziah, Maesaro Maesaroh, Tati Herlina, Young Ho Kim

Status Penulis : ~~Penulis Pertama~~ / Penulis ke 3 / ~~Penulis Korespondensi **)~~

Identitas Jurnal Ilmiah :
a. Nama jurnal : Medicinal Chemistry Research
b. Nomor ISSN : 1054-2523
c. Vol., No., Bulan, Tahun : Vol 26; No 1; November 2016
d. Penerbit : Birkhauser Boston
e. DOI Artikel (jika ada) : 10.1007/s00044-016-1735-3
f. Alamat Web Jurnal : https://link.springer.com
g. Terindeks di : Scopus Q2, SJR 0.352

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c. Kecukupan & kemutakhiran data serta metodologi ..... lele dasar penelitian baik membahas mekanisme 11 senyawa baru dibandingkan senyawa standard kojic Acid dan dihitung nilai IC50 dari 11 senyawa baru. .....

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UNIVERSITAS PRIMA INDONESIA

LEMBAR HASIL PENILAIAN
SEJAWAT SEBIDANG atau *PEER REVIEW*

KARYA ILMIAH : JURNAL ILMIAH

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Nama-nama Penulis	:	Bui Thi Thuy Luyen, Nguyen Phuong Thao, **Wahyu Widowati**, Nurul Fauziah, Maesaro Maesaroh, Tati Herlina, Young Ho Kim
Status Penulis	:	~~Penulis Pertama~~ / Penulis ke 3 / ~~Penulis Korespondensi **)~~
Identitas Jurnal Ilmiah	: a.	Nama jurnal : Medicinal Chemistry Research
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a.	Kelengkapan unsur isi karya	(10%)	4			3,7
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c. Kecukupan & kemutakhiran data serta metodologi
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SEJAWAT SEBIDANG atau *PEER REVIEW*

KARYA ILMIAH : JURNAL ILMIAH

Judul Karya Ilmiah (Artikel)	:	Chemical constituents of Piper aduncum and their inhibitory effects on soluble epoxide hydrolase and tyrosinase
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Status Penulis	:	~~Penulis Pertama~~ / Penulis ke 3 / ~~Penulis Korespondensi **)~~
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b.	Ruang lingkup dan kedalaman pembahasan	(30%)	12			11,65
c.	Kecukupan dan kemutakhiran data/ informasi dan metodologi	(30%)	12			11,7
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b. Ruang lingkup & kedalaman pembahasan .....
Penelitian tentang isolasi & senyawa baru dari Piper aduncum sebagai bahan penghambatan hidrolase dan tirosinase

Ruang lingkup dan kedalaman pembahasan masih belum sepenuhnya memadai

c. Kecukupan & kemutakhiran data serta metodologi. Ide dasar penelitian baik membahas mekanisme 11 senyawa baru dibandingkan senyawa standard kojic Acid dan dihitung nilai IC 50 dari 11 senyawa baru.
Secara umum metodologi sudah memadai dan lengkap

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Reviewer 2

(Prof. Dr. Ermi Girsang, M.Kes)
NIK : 0117057501
UNIVERSITAS PRIMA INDONESIA

Medan,
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