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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Isolation of phenylpropanoid sucrose esters from the roots of *Persicaria orientalis* and their potential as inhibitors of melanogenesis

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Abstract

Cosmetically, hyperpigmentation is an important issue and the most prominent target for inhibiting hyperpigmentation is, tyrosinase, the rate-limiting enzyme in melanogenesis. Therefore, in this research, we have screened several medicinal plants collected from Bangladesh for their anti-tyrosinase activity and found that roots of *Persicaria orientalis* have potent inhibitory activity. Nine compounds, including five phenylpropanoid sucrose esters (**1**–**5**), were isolated from the EtOH extract of *P. orientalis* roots. The chemical structures were determined based on spectroscopic methods. All the tested compounds (**1**–**5**), significantly reduced extracellular melanin formation in B16 melanoma cells and inhibited tyrosinase monophenolase and diphenolase activity in a dose dependent manner. Compound **4** was twice as effective as kojic acid, when L-DOPA was used as the substrate. Compound **1** and **4** showed better inhibitory activity (>59%) on melanin synthesis at a treated concentration of 50 μM compare with arbutin (730 μM). At 6.25 μM concentration, compounds **2** and **5**, inhibited extracellular melanin production by 53.1% and 40.2%, respectively. Structure-activity-relationship suggested that both the feruloyl (C-6′) and acetyl (C-2′/ C-4′) groups in sucrose moiety is essential for cytotoxicity. Our finding indicated the roots of *P. orientalis* is a potential source of natural compounds that could be used in dermatological and cosmetological research. To the best of our knowledge, this is the first study to report the potential melanogenesis inhibitory activity of phenylpropanoid sucrose esters.

Keywords *Persicaria orientalis* · Phenylpropanoid sucrose esters · NMR · melanin · Anti-tyrosinase

Introduction

Cosmetic skin concerns have a major impact on the quality of life of an individual (Salsberg et al. 2016). Abnormal skin pigmentation, apart from causing serious esthetic problems may also lead to life threatening diseases. Melanin, a major pigment in mammalian skin, is produced form melanocytes by the enzymatic oxidation of tyrosine. It is known to protect the skin against harmful effects of ultraviolet (UV) irradiation, oxidative stress, and DNA damage. Moreover, it is also thought to play an important role in the prevention of skin cancer by protecting cells from UV rays. However, it is believed that melanin is also a reason for sunburns and mottle; therefore, compounds inhibiting melanin synthesis are expected to have cosmetic applications as whitening agents (Yamauchi et al. 2011; Baek et al. 2015). Melanin is synthesized in melanosomes and subsequently transferred to the surrounding epidermal keratinocytes (Ando et al. 2007). Melanogenesis is regulated by several melanocyte-specific enzymes such as tyrosinase, tyrosinase-related protein 1 (TRP1), and tyrosinase-related protein 2 (TRP2) (Kobayashi et al. 1994; Yokoyama et al. 1994; Pawelek and Korner 1982). Tyrosinase, a binuclear copper enzyme, is a key regulatory enzyme of melanogenesis. Inhibition of tyrosinase with high efficacy and less adverse side effects, has been a long standing challenge in dermatological and cosmetological sciences (Briganti et al. 2003). Natural products from different biological sources have been extensively utilized in the cosmetic industry because of their lower tendency to cause adverse

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effects and high safety (Kim and Uyama 2005; Parvez et al. 2006; Fuyuno 2004; Chen et al. 2015).

Phenylpropanoid sucrose esters (PSEs) are an important class of plant-derived natural products with great pharmaceutical potential because of their structural diversity and a broad-array of pharmacological and biological activities. PSEs are mostly isolated from plants belonging to the families Rosaceae, Sparganiaceae, Polygonaceae, Rutaceae, Liliaceae, Arecaceae, and Smilacaceae (Gafner et al. 1997; Seidel et al. 1997; Shimomura et al. 1986; Shoyama et al. 1987). The PSEs have antitumor, antioxidant, antidiabetic, neuroprotective, and anti-inflammatory properties (Panda et al. 2011; Takasaki et al. 2001). The number of acetyl moieties plays a useful role in the cytotoxic activities of PSEs (Zhao et al. 2014). Several PSEs have been isolated from different species of Polygonaceae (e.g., *P. lapathifolium, P. perfoliatum*, and *P. hydropiper*) (Narasimhulu et al. 2014). One study identified vanicoside A, B and C from *P. orientalis* using high performance liquid chromatography coupled with mass spectrometry but not purified (Shin et al. 2018).

P. orientalis L. belongs to Polygonaceae family and originated in East Asia, but is now widely distributed throughout Japan. It is used in traditional Chinese medicine to treat various diseases including bacterial infections, tumor, hypertension and cardiomyopathy (Zhong 1999). *P. orientalis*, locally known as Bishkatali in Bangladesh, is an herb widely distributed throughout the country in low-lying areas. It is used in traditional medicine to treat wounds, whooping cough, menorrhagia, fever, and splenic diseases (Medicinal plants of Bangladesh, http://www.mpbd.info). Flavonoids are the major active compounds in *P. orientalis* (Kuroyanagi and Fukushima 1982). Many studies reported the chemical constituents of this plant, including taxifolin, myricitrin, luteolin, protocatechuic acid, apocynin, lappaol B, orientalin, naringenin, quercitrin, kaempferol, β-sitosterol, etc. (Li et al. 2005; Zheng et al. 1997; Philippine Medicinal plants, http://www.stuartxchange.org). Extracts of *P. orientalis* have been found to exhibit important pharmacological effects including anti-diabetic, cardioprotective, osteoblastic, and antioxidant effects (Wei et al. 2009; Nigam et al. 2013; Liao et al. 2013; Xiang et al. 2011). However, to date, there have been no reports on the pharmacological properties of PSEs from *P. orientalis*. Moreover, their effect on melanogenesis has not been thoroughly investigated. In this study, we screened seven different medicinal plants collected from Bangladesh and found that roots of *P. orientalis* have potent tyrosinase inhibition properties. This study aims to describe the isolation and structural elucidation of secondary metabolites from *P. orientalis* roots, as well as the evaluation of their (particularly, PSEs) inhibitory effects on melanogenesis by testing on mushroom tyrosinase and murine B16 cells.

Materials and methods

General experimental procedures

The 1H-NMR (600 MHz) and 13C-NMR (150 MHz) spectra were recorded in chloroform-*d*, acetone-*d6* and methanol-*d4* using a JEOL ECA-600 NMR spectrometer (JEOL, Tokyo, Japan) and chemical shifts (δ) are reported in ppm using tetramethylsilane as an internal standard. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) spectra were measured on a Shimadzu AXIMA Resonance spectrometer (Shimadzu Corporation, Kyoto, Japan). High-performance liquid chromatography (HPLC) analysis was performed using an Inertsil ODS-3 column (4.6 mm $\Phi \times 250$ mm; GL Sciences) equipped with a Jasco MD-2018 Plus photodiode array detector, and preparative HPLC (Shimadzu LC-6AD) was performed using an Inertsil ODS-3 column (20 mm $\Phi \times 250$ mm; GL Sciences). Column chromatography was performed on silica gel (BW-200; Chromatotex) and Sephadex LH-20 (18–111 µm; GE Healthcare). Thin layer chromatography (TLC) was performed on pre-coated silica gel 60 F254 glass plates (Merck, Germany). Visualization of the spots was done using 5% H_2SO_4 (ethanolic) and heat treatment. The other products were commercially available and purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Plant material

P. orientalis L. was collected from Mymensing, Bangladesh in May 2017 and identified by Dr. Syed Hadiuzzaman, former Professor Dept. of Botany, University of Dhaka, Bangladesh. A voucher specimen was designated with the code DUBMB2017-005 and deposited at the Dept. of Biochemistry and Molecular Biology, University of Dhaka, Bangladesh.

Extraction and isolation

Air-dried roots of *P. orientalis* were powdered (~1.5 kg) and extracted with methanol (MeOH) on a rotary shaker for a day at room temperature. This process was repeated thrice. The solvent was removed under reduced pressure to give MeOH extract (105 g), which was successively extracted with hexane, ethyl acetate (EtOAc), and ethanol (EtOH) to

yield hexane extract (7 g), EtOAc extract (11 g), and EtOH extract (25 g), respectively. The EtOH extract (25 g) was subjected to normal-phase silica gel column chromatography (CC) and eluted with gradient conditions of chloroform: MeOH (9:1–1:3 v/v) to give 12 fractions (Fr. E1–E12) by TLC analysis. Fr.E2 (520 mg) was subjected to silica gel CC and eluted with chloroform:MeOH (7:1 v/v) to obtain compound 6 (6.1 mg). Fr.E5 (890 mg) was separated over a Sephadex LH-20 column using MeOH (70% aq) to obtain compound 5 (19 mg). Fr.E6 (1230 mg) was isolated over a Sephadex LH-20 column using MeOH:H_2O (7:3 v/v) and purified by semi-preparative HPLC (Inertsil ODS-3, 10 mm Φ × 250 mm, gradient condition, MeOH:H_2O [6:4–8:2 v/v]) to obtain compound 2 (56 mg). Fr.E7 (1100 mg) was subjected to Sephadex LH-20 CC with MeOH:H_2O (8:2, v/v) and give four subfractions (Fr.E7.1-E7.4). Fr.E7.2 and E7.4 purified by semi-preparative HPLC (gradient condition, MeOH:H_2O [5:5–8:2 v/v]) to obtain compound 3 (45 mg) and compound 4 (21 mg). Fr.E8 (1050 mg) was subjected to Sephadex LH-20 CC with MeOH:H_2O (7:3, v/v) to obtain five subfractions (Fr.E8.1-E8.5). Fr.E8.1 and E8.5 were purified by semi-preparative HPLC to obtain compound 9 (5.1 mg) and compound 1 (19 mg). Compound 7 (3.2 mg) and 8 (12 mg) were obtained from Fr.E9 by Sephadex LH-20 CC with MeOH and purified by semi-preparative HPLC.

Compound 1 (Hydropiperoside)

Colorless amorphous powder; UV (MeOH): λ_{max}: 228 and 312 nm; ^{1}H NMR (600 MHz, Acetone-D_6): δH (ppm): *fructose*: 4.28 (1H, s, H-1), 5.60 (1H, d, $J = 8.2$ Hz, H-3), 4.61 (1H, m, H-4), 4.22 (1H, t, $J = 7.56$ Hz, H-5), 4.52–4.56 (2H, m, H-6), *glucose*: 5.50 (1H, d, $J = 7.5$ Hz, H-1′), 3.38 (1H, m, H-2′), 3.68 (1H, t, $J = 9.6$ Hz, 8.9 Hz, H-3′), 4.04 (1H, m, H-5′), 3.92 (1H, d, $J = 11.7$ Hz, H_a-6′), 3.77 (1H, dd, $J = 6.8$ Hz, H_b-6′), *trans-p-coumaroyl moiety*:7.49, 7.54 (2H, 4H, d, $J = 8.2$ Hz, H-2″/6″ - H-2‴/6‴), 6.83, 6,86 (2H, 1H, d, $J = 8.2$ Hz, 7.6 Hz, H-3″/5″- H-3‴/5‴), 7.63 (2H, dd, $J = 6.8$ Hz, H-7′′, H-7‴), 7.71 (1H, d, $J = 15.8$ Hz, H-7‴), 6.43 (1H, s, H-8″), 6.37 (2H, d, $J = 9.0$ Hz, H-8′′, H-8‴); ^{13}C NMR (150 MHz, Acetone-D_6): δC (ppm): *fructose*: 64.8 (C-1), 102.4 (C-2), 77.8 (C-3), 72.9 (C-4), 80.1 (C-5), 64.7 (C-6); *glucose*: 92.2 (C-1′), 71.3 (C-2′), 74.2 (C-3′), 72.1 (C-4′), 73.2 (C-5′), 62.4 (C-6′), *trans-p-coumaroyl moiety*: 126.07 × 2, 126.12(C-1″– C-1‴), 130.25 × 2, 130.3 × 2, 130.5 × 2 (C-2″/6″– C-2‴/6‴), 115.89 × 2, 115.91 × 2, 115.94 × 2 (C-3″/5″– C-3‴/5‴), 159.89, 159.92, 160.0 (C-4″– C-4‴), 145.1 × 2, 145.9 (C-7″– C-7‴), 114.0, 114.4 × 2 (C-8″– C-8‴), 166.1,166.2, 166.8 (C-9″– C-9‴). ESI-MS *m/z* 803 $[M+Na]^+$, corresponding to the molecular formula $C_{39}H_{40}O_{17}$. All data were comparable to the published data (Fukuyama et al. 1983).

Compound 2 (Vanicoside A)

Colorless amorphous powder; UV (MeOH): λ_{max}: 228 and 316 nm; ^{1}H NMR (600 MHz, CD_3OD): δH (ppm): *fructose*: 4.17 (1H, m, H-1a), 4.67 (1H, m, H-1b), 5.54 (1H, d, $J = 8.2$ Hz, H-3), 4.57 (1H, m, H-4), 4.17 (1H, m, H-5), 4.49 (2H, m, H-6), *glucose*: 5.68 (1H, d, $J = 3.4$ Hz, H-1′), 4.65 (1H, m, H-2′), 3.86 (1H, m, H-3′), 3.39 (1H, t, $J = 9.0$ Hz & 9.6 Hz, H-4′), 4.21 (1H, m, H-5′), 4.27 (2H, m, H-6′), *feruloyl moiety*: 7.18 (1H, d, $J = 7.6$ Hz, H-2″), 7.68 (1H, m, H-4″), 6.79 (1H, m, H-5″), 7.10 (1H, t, $J = 8.2$ Hz & 6.8 Hz, H-6″), 7.71 (1H, d, $J = 6.2$ Hz, H-7″), 6.47 (1H, d, $J = 5.5$ Hz, H-8″), 3.86 (3H, s, O-Me), *trans-p-coumaroyl moiety*: 7.33 (2H, d, $J = 8.2$ Hz, H-2‴/6‴), 7.39 (2H, d, $J = 8.2$ Hz, H-2′′′′/6′′′′), 7.48 (2H, d, $J = 8.2$ Hz, H-2′′′′′/6′′′′′), 6.72-6.75 (6H, m, H-3‴/5′′′ - H3′′′′/5′′′′), 7.69 (1H, s, H-7′′′), 7.60 (1H, d, $J = 2.6$ Hz, H-7′′′′), 7.58 (1H, d, $J = 3.5$ Hz, H-7′′′′′), 6.45 (1H, d, $J = 5.5$ Hz, H-8′′′), 6.34 (1H, d, $J = 15.8$ Hz, H-8′′′′), 6.27 (1H, d, $J = 15.8$ Hz, H-8′′′′′), *acetyl moiety*: 2.07(1H, s, CO-CH_3); ^{13}C NMR (150 MHz, CD_3OD): δC (ppm): *fructose*: 64.1 (C-1), 102.2 (C-2), 78.1 (C-3), 72.9 (C-4), 79.7 (C-5), 64.1 (C-6), *glucose*: 89.3 (C-1′), 72.9 (C-2′), 70.9 (C-3′), 70.8 (C-4′), 71.0 (C-5′), 65.0 (C-6′), *feruloyl moiety*: 125.7(C-1″), 110.0 (C-2″), 149.2(C-3″), 147.9 (C-4″), 115.0 (C-5″), 123.2(C-6″), 145.57 (C-7″), 113.24 (C-8″), 167.0(C-9″), 55.12 (O-Me), *trans-p-coumaroyl moiety*: 125.81, 126.3, 126.4(C-1‴– C-1′′′′′), 129.9 × 2, 129.9 × 2, 130.2 × 2 (C-2‴/6‴– C-2′′′′′/6′′′′′), 115.1 × 2, 115.4 × 2, 115.5 × 2 (C-3‴/5‴– C-3′′′′′/5′′′′′), 159.9, 160.0, 160.2 (C-4‴– C-4′′′′′), 145.9, 146.7 (C-7‴, C-7′′′′′), 113.5, 113.9, 114.5 (C-8‴– C-8′′′′′), 167.5, 167.8 (C-9‴, C-9′′′′′), *acetyl moiety*: 19.7 (CO-CH_3) and 171.1 (CO-CH_3). ESI-MS *m/z* 1021 $[M+Na]^+$, corresponding to the molecular formula $C_{51}H_{50}O_{21}$. All data were comparable to the published data (Zimmermann and Sneden 1994).

Compound 3 (Vanicoside B)

Colorless amorphous powder; UV (MeOH): λ_{max}: 232 and 316 nm; ^{1}H NMR (600 MHz, Acetone-D_6): δH (ppm): *fructose*: 4.26/4.29 (2H, m, H-1), 5.63 (1H, d, $J = 8.2$ Hz, H-3), 4.71 (1H, m, H-4), 4.25 (1H, m, H-5), 4.55/4.61 (2H, m, H-6), *glucose*: 5.53 (1H, d, $J = 3.4$ Hz, H-1′), 4.33 (1H, m, H-2′), 3.69 (1H, t, $J = 8.2$ Hz & 8.9 Hz, H-3′), 3.34 (1H, s, H-4′), 3.36 (1H, t, $J = 9.6$ Hz & 8.9 Hz, H-5′), 4.22/4.70 (2H, m, H-6′), *feruloyl moiety*: 7.33 (1H, s, H-2″), 6.84 (1H, d, $J = 3.4$ Hz, H-5″), 7.09 (1H, d, $J = 8.2$ Hz, H-6″), 7.63 (1H, d, $J = 4.1$ Hz, H-7″), 6.40 (1H, d, $J = 6.8$ Hz, H-8″), 3.85(3H, s, O-Me), *trans-p-coumaroyl moiety*: 7.56 (2H, d, $J = 8.9$ Hz, H-2′′/6′′), 7.50 (2H, d, $J = 8.9$ Hz, H-2′′′′/6′′′′), 7.48 (2H, d, $J = 8.9$ Hz, H-2′′′′′/6′′′′′), 6.81–6.86 (6H, m, H-3′′/5′′ - H3′′′′/5′′′′), 7.48 (1H, d, $J = 8.9$ Hz, H-6′′), 7.69 (1H, s, H-7′′), 6.32–6.54 (3H, m, H-8′′ - 8′′′′); ^{13}C NMR

(150 MHz, Acetone-D_6): δC (ppm): *fructose*: 64.8 (C-1), 102.2 (C-2), 77.8 (C-3), 73.1 (C-4), 80.1 (C-5), 64.6 (C-6), *glucose*: 91.8 (C-1′), 71.1 (C-2′), 74.2 (C-3′), 71.9 (C-4′), 71.2 (C-5′), 64.4 (C-6′), *feruloyl moiety*: 126.1 (C-1″), 110.4 (C-2″), 147.9 (C-3″), 149.2 (C-4″), 115.9 (C-5″), 123.4(C-6″), 145.1 (C-7″), 114.9 (C-8″), 166.8 (C-9″), 55.5 (O-Me), *trans-p-coumaroyl moiety*: 126.1, 126.14, 126.16 (C-1‴– C-1⁗), 130.22 × 2, 130.24 × 2, 130.5 × 2 (C-2‴/6‴– C-2⁗/6⁗), 115.9 × 2, 115.8 × 2, 115.1 × 2 (C-3‴/5‴– C-3⁗/5⁗), 159.80, 159.83, 160.0 (C-4‴– C-4⁗), 145.1 × 2, 145.9 (C-7‴– C-7⁗), 114.9, 114.3, 113.9 (C-8‴– C-9⁗), 166.6, 166.1, 166.0 (C-9‴– C-9⁗). ESI-MS *m/z* 979 $[M+Na]^+$, corresponding to the molecular formula $C_{49}H_{48}O_{20}$. All data were comparable to the published data (Kiem et al. 2008).

Compound 4 (Vanicoside C)

Yellow amorphous powder; UV (MeOH): λ_{max}: 232 and 316 nm; ^{1}H NMR (600 MHz, Acetone-D_6): δH (ppm): *fructose:* 4.28 (2H, d, $J = 11.6$ Hz, H-1), 5.56 (1H, d, $J = 8.2$ Hz, H-3), 4.59 (1H, d, $J = 3.42$ Hz, H-4), 4.20 (1H, m, H-5), 4.50 (1H, d, $J = 4.80$ Hz, H_a-6), 4.10 (1H, d, $J = 10.98$ Hz, H_b-6), *glucose*: 5.61 (1H, d, $J = 3.42$ Hz, H-1′), 4.61 (1H, d, $J = 3.4$ Hz, H-2′), 3.90 (1H, d, $J = 7.5$ Hz, H-3′), 3.50 (1H, t, $J = 9.6$ Hz & 8.9 Hz, H-5′), 3.92 (1H, d, $J = 7.56$ Hz, H-6′), *trans-p-coumaroyl moieties*: 7.51, 7.55 (2H, 4H, d, $J = 8.2$ Hz, H-2‴/6″–H-2⁗/6⁗), 6.83 - 6.86 (6H, m, H-3″/5″- H3⁗/5⁗), 7.59 (1H, d, $J = 8.9$ Hz, H-7‴), 7.66 (1H, d, $J = 4.08$ Hz, H-7″), 7.73 (1H, d, $J = 3.42$ Hz, H-7⁗), 6.38 (1H, d, $J = 4.08$ Hz, H-8″), 6.41 (1H, d, $J = 3.48$ Hz, H-8‴), 6.48 (1H, d, $J = 16.0$ Hz, H-8⁗), acetyl moieties: 2.05 (3H, 2′-COCH3); ^{13}C NMR (150 MHz, Acetone-D_6) δC (ppm); *fructose:* 64.8 (C-1), 102.4 (C-2), 77.9 (C-3), 73.2 (C-4); 80.1 (C-5), 64.6 (C-6); *glucose*: 89.6 (C-1′), 73.2 (C-2′), 71.0 (C-3′), 71.1 (C-4′), 71.9 (C-5′), 62.0 (C-6′), *trans-p-coumaroyl moieties*: 126.09 × 2, 126.2(C-1‴– C-1⁗), 130.26 × 2, 130.27 × 2, 130.5 × 2 (C-2″/6″– C-2⁗/6⁗), 114.9 × 2, 115.8 × 2, 115.9 × 2 (C-3″/5″– C-3⁗/5⁗), 159.8, 159.9, 160.0 (C-4″– C-4⁗), 145.0, 145.2, 145.9 (C-7″– C-7⁗), 113.9, 114.2, 114.4 (C-8″– C-8⁗), 166.1,166.2, 166.6 (C-9″ – C-9⁗), acetyl moieties: 170.3 (2′-COCH3). ESI-MS *m/z* 845 $[M+Na]^+$, corresponding to the molecular formula $C_{41}H_{42}O_{18}$. All data were comparable to the published data (Brown et al. 1998).

Compound 5 (Vanicoside E)

Colorless amorphous powder, UV (MeOH): λ_{max}: 232 and 316 nm; ESI-MS: ^{1}H-NMR (600 MHz, Acetone-D_6): δH (ppm): *fructose:* 4.06 (2H, m, H-1), 5.56 (1H, d, $J = 7.5$ Hz, H-3), 4.63 (1H, m, H-4), 4.23 (1H, m, H-5), 4.58 (2H, m, H-6), *glucose*: 5.71 (1H, dd, $J = 4.2$ Hz, H-1'), 4.78 (1H, d, $J = 7.2$ Hz, H-2), 4.91 (1H, d, $J = 9.6$ Hz, H-5'), 4.44 (2H, m, H-6′), *feruloyl moiety*: 7.32 (1H, d, $J = 2.8$ Hz, H-2″), 6.89 (1H, d, $J = 8.4$ Hz, H-5″), 7.1 (d, $J = 11.9$ Hz, H-6″), 6.33 (1H, d, $J = 15.8$ Hz, H-8″), 3.86 (3H, s, O-Me), *trans-p-coumaroyl moieties*: 7.49, 7.60, 7.78 (2H, 2H, 2H, d, $J = 8.2$ Hz, H-2‴/6‴–H-2⁗/6⁗), 6.81–6.87 (6H, m, H-3‴/5‴- H3⁗/5⁗), 7.58, 7.64, 7.71 (1H, 1H, 1H, d, $J = 8.9$ Hz, 11.0 Hz & 15.8 Hz, H-7‴–H-7⁗), 6.41, 6.46, 6.50 (1H, 1H, 1H, d, $J = 16.0$ Hz, 8.2 Hz, & 15.8 Hz, H-8‴ –H-8⁗), and *acetyl moieties*: 2.05 (3H, 2′-$COCH_3$) and 1.96 (3H, 4′-$COCH_3$); ^{13}C-NMR (150 MHz, Acetone-D_6): δC (ppm): *fructose*: 68.8 (C-1), 102.6 (C-2), 80.2 (C-3), 73.3 (C-4), 80.4 (C-5), 63.1 (C-6), *glucose*: 89.3 (C-1'), 72.7 (C-2′), 72.5 (C-3′), 73.1 (C-4′), 71.4 (C-5'), 68.6 (C-6'), *feruloyl moiety*: 126.6 (C-1″), 113.7 (C-2"), 147.9 (C-3″), 149.3 (C-4″), 116.0 (C-5″), 123.5 (C-6″), 145.1 (C-7″), 114.1 (C-8″), 167.0 (C-9″), 55.5 (O-Me), *trans-p-coumaroyl moieties*: 125.9, 126.0, 126.3 (C-1‴– C-1⁗), 130.5 × 2, 130.3 × 2, 130.2 × 2 (C-2‴/6‴– C-2⁗/6⁗), 115.2 × 2, 115.3 × 2, 115.9 × 2 (C-3‴/5‴– C-3⁗/5⁗), 159.8 × 2, 159.9 (C-4‴– C-4⁗), 145.3, 145.4, 145.5 (C-7‴– C-7⁗), 114.3, 114.4, 114.6 (C-8‴– C-8⁗), 166.0, 166.1, 166.5 (C-9‴– C-9⁗), and *acetyl moieties*: 20.1 (2′-$COCH_3$), 170.1 (2′-$COCH_3$), 20.0 (4′-$COCH_3$), and 169.6 (4′-$COCH_3$). ESI-MS *m/z* 1063 $[M+Na]^+$ and 1039 $[M-H]^-$, corresponding to the molecular formula $C_{53}H_{52}O_{22}$. All data were comparable to the published data (Kiem et al. 2008).

Compound 6 (Exoticin)

Pale yellow needles. UV (MeOH): λ_{max}: 288 and 332 nm; ^{1}H NMR (600 MHz, CD_3OD): δH (ppm) = 7.52 (2H, s, H-2′/ H-6′), 4.08 (3H, s, 7-OMe), 3.99 (3H, 8-OMe), 3.91 (6H, s, 3′-OMe, 5′-OMe), 3.90 (3H, s, 6-OMe), 3.89 (3H, s, 5-OMe), 3.84 (3H, s, 4'-OMe), 3.83 (3H, s, 3-OMe); ^{13}C NMR (150 MHz, CD_3OD): δC (ppm) = 174.4 (C-4), 153.6 (C-2), 153.2 (C-3′ and C-5′), 151.9 (C-7), 147.8 (C-5), 146.9 (C-9), 141.0 (C-6), 140.9 (C-4′), 138.0 (C-8), 125.8 (C-1′), 114.4 (C-10), 105.6 (C-2′ and C-6′), 61.3 (5-OMe), 61.2 (8-OMe), 60.9 (7-OMe), 60.8 (6-OMe), 59.9 (4′-OMe), 59.1 (3-OMe), 55.4 (3′-OMe and 5′-OMe); ESI-MS *m/z* 462.9 $[M+H]^+$, corresponding to the molecular formula $C_{23}H_{26}O_{10}$. All data were comparable to the published data (Shajib et al. 2017).

Compound 7 (EpiCatechin-3-o-gallate)

Pale red solid; UV (MeOH): λ_{max}: 204 and 276 nm; ^{1}H NMR (600 MHz, CD_3OD): δH (ppm) 5.01 (1H, s, H-2), 5.50 (1H, br s, H-3), 2.96, 2.82 (1H, dd, $J = 4.8$ Hz & 4.9 Hz, H-4), 5.94 (1H, s, H-5), 5.93 (1H, s, H-6), 6.90 (1H, d, $J = 2.1$ Hz, H-2′), 6.67 (1H, d, $J = 8.2$ Hz, H-5′), 6.78 (1H,

d, $J = 8.2$ Hz, H-6′), 6.92 (1H, s, H-2′′), 6.92 (1H, s, 6″); ^{13}C NMR (150 MHz, CD_3OD): δC (ppm) 77.3 (C-2), 68.6 (C-3), 25.5 (C-4), 98.0 (C-4a), 95.15 (C-5), 94.1 (C-6), 156.6 (C-7), 156.5 (C-8), 155.9 (C-8a), 130.1 (C-1′), 113.8 (C-2′), 144.6(C-3′), 144.9 ((C-4′), 114.6 (C-5′),118.0 (C-6′), 120.0 (C-1″), 108.6 (C-2″), 144.9 (C-3″) 138.5 (C-4″), 144.9 (C-5″), 108.8 (C-6″), 166.3 (CO). ESI-MS *m/z* 442 $[M-H]^+$, corresponding to the molecular formula $C_{22}H_{18}O_{10}$. All data were comparable to the published data (Davis et al. 1996).

Compound 8 (Gallic acid)

Colorless solid; UV (MeOH): λ_{max}: 216 nm and 272 nm; ^{1}H NMR (600 MHz, CD_3OD): δH (ppm) 7.02 (1H, s, H-2); ^{13}C NMR (150 MHz, CD_3OD): δC (ppm) 121.0 (C-1), 108.95 (C-2 & C-6), 145.0 (C-3 & C-5), 138.1 (C-4), 169.3 (C-7; C = O). ESI-MS *m/z* 171 $[M+H]^+$, corresponding to the molecular formula $C_7H_6O_5$. All data were comparable to the published data (Kamatham et al. 2015).

Compound 9 (3,4-dihydroxy benzoic acid)

Colorless solid; UV (MeOH): λ_{max}: 204, 220, 260 and 296 nm; ^{1}H NMR spectra (600 MHz, Acetone-D_6): δH (ppm) 7.50 (1H, d, $J = 1.4$ Hz, H-2), 6.86 (1H, d, $J = 8.2$ Hz, H-5), 7.44 (1H, dd, $J = 3.6$ Hz & 2.0 Hz, H-6); ^{13}C NMR (150 MHz, Acetone-D_6): δC (ppm) 122.3 (C-1), 116.6 (C-2), 144.7 (C-3), 149.9 (C-4), 114.9 (C-5), 122.7 (C-6), 166.8 (C-7, COOH). ESI-MS *m/z* 177 $[M+Na]^+$ and 154$[M-H]^+$, corresponding to the molecular formula $C_7H_6O_4$. All data were comparable to the published data (Guria et al. 2013).

Tyrosinase activity assay

The tyrosinase activity assay was performed with minor modifications using a previously described method (Batubara et al. 2010; Batubara et al. 2011). The extract (60 µl) was placed in a 96-well plate and 30 µl of mushroom tyrosinase (333 U/mL in phosphate buffer, 50 mM, pH 6.5) and 110 µl of substrates (2 mM L-tyrosine or 2 mM L-DOPA) were added. After incubation at 37 °C for 15 min, the absorbance was measured at 510 nm using a microplate reader. The tyrosinase activity is expressed as the half-maximal inhibitory concentration (IC_{50}), which is the concentration of the samples producing 50% inhibition.

Cell culture

The mouse melanoma B16-F0 cells (DS Pharma Biomedical, Osaka, Japan) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 9% fetal bovine serum, 78 mg/L penicillin, and 111 mg/L streptomycin at 37 °C in a humidified atmosphere of 5% CO_2.

Measurement of cellular melanin content

The measurement of cellular melanin was performed according to a previously described method (Yamauchi et al. 2014) with minor modifications. In brief, confluent cultures of B16 melanoma cells were rinsed in phosphate-buffered saline (PBS) and detached using 0.25% trypsin/ethylenediaminetetraacetic acid (EDTA). The cells were placed in a 24-well plate (5.0×10^4 cells/well) and allowed to adhere at 37 °C for 24 h. Samples were added to the cells followed by incubation for 72 h, and then 200 µl of the culture medium was added to a 96-well plate. The extracellular melanin content was determined by measuring the absorbance of the medium at 510 nm using a microplate reader. The cells were then washed with PBS followed by lysis in 600 µl of 1 M sodium hydroxide (NaOH) with heating at 100 °C for 30 min to solubilize the melanin. The resulting lysate (250 µl) was placed in a 96-well microplate, and the intracellular melanin content was determined by measuring the absorbance at 405 nm using a microplate reader. Each experiment was repeated thrice. The melanin production activity is expressed as a percentage of that of the control cells treated with dimethyl sulfoxide (DMSO) alone.

Cell viability

Cell viability measurement was performed according to a previously described method (Arung et al. 2011), using the microculture tetrazolium (MTT) assay. Cells were cultured in 24-well plates at a density of 5.0×10^4 cells/well. After incubation, 50 µl of the MTT reagent (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide in PBS, 5 mg/mL) was added to each well. The plates were incubated in a humidified atmosphere of 5% CO_2 at 37 °C for 4 h. After the medium was removed, 1.0 mL isopropyl alcohol (containing 0.04 N hydrochloric acid, HCl) was added to each well to dissolve the formazan crystals that formed, and 150 µl was transferred to a 96-well plate. The absorbance was measured at 590 nm using a microplate reader. Each experiment was performed in triplicate. The cell viability is expressed as a percentage of that of the control cells treated with the DMSO vehicle alone.

Statistical analysis

Cells experiments were performed in triplicate ($n = 3$) and data are expressed as mean values and standard deviation. Statistical significance was evaluated using the Student's

t test. A *p*-value of <0.05 was considered statistically significant.

Results and discussion

We screened seven medicinal plants (MeOH extracts) collected from Bangladesh for their tyrosinase inhibitory activity and found that only *P. orientalis* possesses potent anti-tyrosinase activity with an IC_{50} values of 13 and 17 µg/mL (Table 1). Roots of *P. orientalis* were extracted with MeOH and partitioned into n-hexane, EtOAc, and EtOH fractions, respectively. The anti-tyrosinase effects of different extracts were evaluated by measuring their potential to inhibit mushroom tyrosinase using L-tyrosine and L-DOPA as substrates. EtOH extracts exhibited promising tyrosinase monophenolase and diphenolase inhibitory activity with an IC_{50} values of 22 and 39 µg/mL, respectively, compared to Kojic acid, used as a control (Table 2). To isolate the active components, the EtOH extracts were subjected to silica gel and Sephadex LH-20 CC followed by C-18 reversed-phase HPLC to obtain nine compounds (**1–9**) (Fig. 1). Among them five were PSEs (compounds **1–5**) and the major secondary metabolites of the root of *P. orientalis*. Compounds **1–9** were identified from the detailed analysis of their 1D, 2D NMR and ESI mass spectroscopic data in comparison with data reported in the literature. Compounds **1–5** were purified from *P. orientalis* for the first time.

Table 1 Tyrosinase inhibitory activities of MeOH extracts of different medicinal plants collected from Bangladesh

Plants name	L-Tyrosine IC_{50} (µg/mL)	L-DOPA IC_{50} (µg/mL)
Tectona grandis (L)	>1000	>1000
Coriandrum sativum (W)	>1000	>1000
Persicaria orientalis (L)	903	>1000
Persicaria orientalis (R)	13	17
Trigonella foenum-graecum (w)	>1000	>1000
Calotropis gigantean (L)	>1000	>1000
Calotropis gigantean (S)	>1000	>1000
Calotropis gigantean (R)	>1000	>1000
Amorphophallus campanulatus (TR)	339	>1000
Allamanda (L)	441	>1000
Kojic acid	2	26

L leaf, *R* root, *S* stem, *W* whole plant, *TR* tuber root

Table 2 Tyrosinase inhibitory activities of different extracts prepared from *P. orientalis* root

Extracts	L- Tyrosine IC_{50} (µg/mL)	L-DOPA IC_{50} (µg/mL)
n-Hexane	>1000	>1000
Ethyl Acetate	80	311
Ethanol	22	39
Kojic acid	2	26

The phenylpropanoid pathway in plants is responsible for the biosynthesis of a huge amount of secondary metabolites derived from phenylalanine and tyrosine. Phenylpropanoid compounds and their glycosides are produced by the shikimate pathway. Glycosylation can change phenylpropanoid solubility, stability, and toxicity. It also influences compartmentalization and biological activity (Waterman and Mole 1994; Le Roy et al. 2016). The phenylpropanoid glycosides such as vanicosides have been found in many plants belonging to the families Polygonaceae, Rosaceae, Brassicaceae, Bignoniaceae, Ballotaceae, and Liliaceae. Vanicoside and vanicoside-related substances have been reported to exhibit several pharmacological activities including antioxidant, cytotoxic, protein kinase C inhibitory, anti-tumor, anti-inflammatory, and antimicrobial activities (Fukuyama et al. 1983; Zimmermann and Sneden 1994; Kiem et al. 2008; Brown et al. 1998). One study reported that plant-derived phenylpropanoid compounds and their synthesized glycosides have the potential for practical application as whitening cosmetic agents (Tanimoto et al. 2006). However, there have been no reports on the anti-melanogenic activity of vanicosides. The present report is the first to demonstrate the inhibition of mushroom tyrosinase activity and melanin production in B16 melanoma cells by vanicoside A, B, C, E, and hydropiperoside isolated from the roots of *P. orientalis*.

Hyperpigmentary disorders, responsible for overproduction of melanin, are a common cosmetic concern globally. Melanogenesis is regulated by several melanocyte-specific enzymes such as tyrosinase, tyrosinase-related protein 1 (TRP1), and tyrosinase-related protein 2 (TRP2) (Kobayashi et al. 1994; Yokoyama et al. 1994), with tyrosinase being the key regulatory enzyme of melanogenesis. Targeting tyrosinase inhibition, with high efficacy and less adverse side effects, is an obvious target in dermatological and cosmetological research for the prevention of hyperpigmentation. To examine the effect of isolated PSEs on the activity of mushroom tyrosinase, L-tyrosine and L-DOPA were used as substrates with kojic acid as the positive control (An et al. 2005). Isolated compounds **1–5** were evaluated for their whitening effects by testing their inhibition of mushroom tyrosinase activity (Table 3). All five compounds showed promising tyrosinase monophenolase and diphenolase inhibitory activity. Using L-tyrosine as a substrate, compound **1** showed strongest inhibition of tyrosinase activity (IC_{50} value 27.1 µM), with a potency of approximately half of that of kojic acid, a well-known inhibitor of tyrosinase (IC_{50} value 14.15 µM). Compounds **2** and **4** inhibit tyrosinase monophenolase activity with IC_{50} values of 37.29 and 39.0 µM, respectively. Feruloyl sucrose esters (3,6-diferuloyl-3′,6′-diacetyl

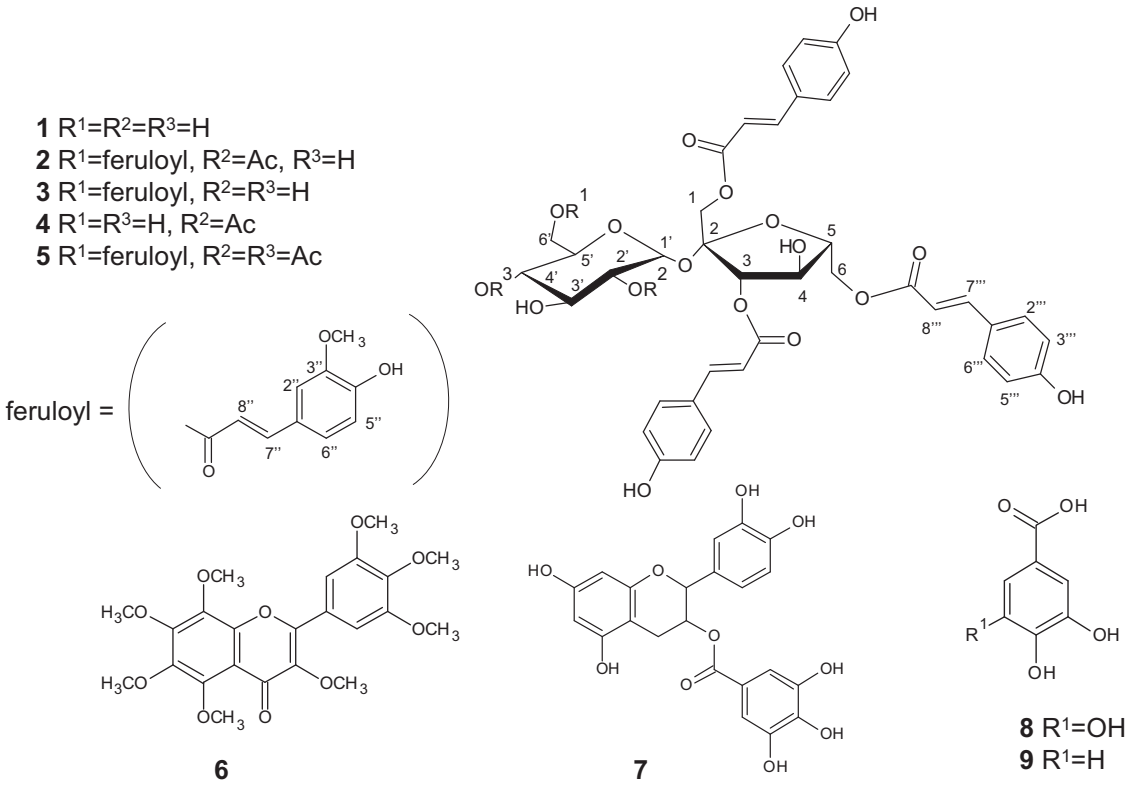

Fig. 1 Structures of isolated compounds **1**–**9** from root of *Persicaria orientalis*

Table 3 Tyrosinase inhibitory activities of compounds **1**–**5** isolated from *P. orientalis* root

Compounds	L- Tyrosine IC_{50} (μM)	L-DOPA IC_{50} (μM)
1	27.1	166.15
2	37.29	135.91
3	62.0	113.13
4	39.0	91.38
5	45.23	189.96
Kojic acid	14.15	181.40

sucrose and smilaside A) isolated from *Oryza sativa* roots inhibit tyrosinase monophenolase activity with an IC_{50} value of 47.33 and 45.13 μM, respectively (Cho et al. 2015), which also supports our findings. Compounds **1**–**4** exhibited better diphenolase inhibitory activity with IC_{50} values of 166.15, 135.9, 113.1, and 91.38 μM, respectively, as compared to kojic acid, used as a positive control (IC_{50} value 181.4 μM). Compound **4** was twice as effective as kojic acid, when L-DOPA was used as the substrate. This result is also reflected by the decrease in extracellular melanin formation by compounds **1**–**5** (Table 4).

To ascertain the depigmentation activities of compounds **1**–**5**, the inhibitory effects on melanin synthesis were evaluated in B16 cells. The compounds were applied to these cells at concentrations of 50, 25, 12.5, and 6.25 μM for three days, and cell viability was assessed by MTT assay. The results for extracellular and intracellular melanogenic activity of compounds **1**–**5** are presented in Table 4. Our previous study suggested that extracellular melanin contents in control wells was 23 times more than intracellular melanin (Yamauchi et al. 2015). Compounds **1** and **4** showed non-cytotoxic effects at all tested concentrations and compound **3** was slightly toxic at high concentration. In contrast, compounds **2** and **5** were found to be toxic to B16 cells at all tested concentrations except 6.25 μM. This can be attributed to the addition of feruloyl and acetyl groups in the glucose moiety. As shown in Table 4, all the compounds have an inhibitory effect on melanin synthesis in a dose-dependent manner. Notably, compound **3** showed a significantly higher extracellular (71.9% and 72.0%) and intracellular (35.3 and 27.3%) melanin inhibitory activity at 50 and 25 μM, respectively. Compounds **1** and **4** inhibited extracellular melanin production by 59.6% and 59.5%, at a concentration of 50 μM without affecting cell viability. Compounds **2** and **5**, which are non-toxic at 6.25 μM to B16 cells, inhibited extracellular melanin production by 53.1 and 40.2%, respectively at the same concentration. It has been found that arbutin, a whitening agent, exhibits an inhibitory effect of 58% on melanin synthesis at 730 μM. Compared with arbutin, compounds **1, 3**, and **4** showed better

Table 4 Intra- and extracellular melanogenesis activities and cell viabilities of compounds **1**–**5**, isolated from root of *P. orientalis*

Compound name and concentration (μM)	Cell viability (%)	Intracellular melanogenesis activity (%)	Extracellular melanogenesis activity (%)
Hydropiperoside			
50	105 ± 1.7	88 ± 2.3*	40 ± 1.3**
25	97 ± 5.4	97 ± 2.9	59 ± 14.8**
12.5	101 ± 14.1	104 ± 1.9	73 ± 1.1**
6.25	102 ± 20.8	103 ± 4.2	91 ± 1.2*
Vanicoside A			
50	5 ± 0.3**	59 ± 1.5**	29 ± 0.68**
25	5 ± 0.2**	56 ± 1.3**	26 ± 11.1**
12.5	47 ± 4.8**	64 ± 1.5**	26 ± 0.84**
6.25	122 ± 3.5**	146 ± 6.7**	46 ± 12.5*
Vanicoside B			
50	75 ± 2.5*	64 ± 1.2**	28 ± 0.54**
25	79 ± 2.3*	72 ± 3.1**	28 ± 7.0**
12.5	78 ± 2.9*	89 ± 1.3**	48 ± 3.2**
6.25	84 ± 2.2	97 ± 0.57	76 ± 5.3**
Vanicoside C			
50	90 ± 2.1	115 ± 2.3**	40 ± 0.56**
25	96 ± 5.7	124 ± 1.5**	62 ± 11.9*
12.5	115 ± 4.2	121 ± 4.8**	91 ± 4.4
6.25	129 ± 4.0*	120 ± 0.98**	112 ± 8.6
Vanicoside E			
50	5.2 ± 0.12**	58 ± 1.1**	24 ± 0.41**
25	5.4 ± 0.25**	57 ± 0.37**	24 ± 12.8**
12.5	44 ± 3.2**	71 ± 4.7**	23 ± 1.2**
6.25	125 ± 7.3**	100 ± 21.6	59 ± 8.1*
Arbutin			
730	99.2 ± 2.2	109 ± 3.9*	42 ± 1.2**

All data were expressed as the mean ± S.D. Differences were examined for statistical significance using Student's *t*-test. $n = 3$. $^{*}p < 0.05$ compared with respective control values. $^{**}p < 0.01$ compared with respective control values

inhibitory activity on melanin synthesis even at a fifteen fold lower concentration of 50 μM (Table 4).

A comparison of the chemical structures showed that the presence of a hydroxyl group on the aromatic ring may be essential to inhibit tyrosinase activity, as most tyrosinase inhibitors have a specific hydroxyl group on the aromatic ring (Chang 2009). This is also supported by the finding that the hydroxyl group in the benzene ring is required for improved tyrosinase-inhibitory activity of phenylpropanoid compounds (Tanimoto et al. 2006). The results showed a structure-activity-relationship (SAR) where the presence of the feruloyl group at C-6′ and the acetyl group at C-2′ and C-4′ of sucrose increases cytotoxicity to B16 melanoma cells. A study suggested that 6′-feruloylated PSEs had marked cytotoxicity in vitro (Zheng et al. 2012), but our findings suggested that both the feruloyl (C-6′) and acetyl (C-2′/ C-4′) groups in the sucrose moiety are essential for cytotoxicity. Feruloyl group at the C-6′ of sucrose showed slight toxicity in B16 cells. However, neither feruloyl (C-6′) nor acetyl (C-2′) groups alone, in the sucrose moiety, exhibited cytotoxicity. Further research on *P. orientalis* root extract and isolated PSEs is necessary to fully understand their therapeutic potential as whitening cosmetic agents.

Conclusion

The present phytochemical investigation of *P. orientalis* roots resulted in the purification of five known PSEs for the first time, of which compounds **1** and **5** first reported from this plant. We evaluated the cytotoxicity and the inhibitory activity of these compounds on tyrosinase and melanin synthesis with a broader objective of using them as whitening agents in cosmetics. All tested compounds significantly reduced extracellular melanin formation in B16 melanoma cells and inhibited tyrosinase monophenolase and diphenolase activity. Among them, compound **1** and **4** were the best candidates to use as whitening agents in cosmetics without negatively affecting cell viability. Taxifolin, a known tyrosinase inhibitor, was also isolated from this plant (Wei et al. 2009). Hence, the root extract of *P. orientalis* has several promising compounds that could be used as skin-whitening agents. However, additional investigation is required to elucidate the mechanism of action of these compounds. To the best of our knowledge, this is the first study to report the potential melanogenesis inhibitory activity of compounds **1**–**5** from *P. orientalis* root extract.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Ando H, Kondoh H, Ichihashi M, Hearing VJ (2007) Approaches to identify inhibitors of melanin biosynthesis via the quality control of tyrosinase. J Investig Dermatol 127:751–761

An BJ, Kwak JH, Park JM, Lee JY, Park TS, Lee JT, Son JH, Jo C, Byun MW (2005) Inhibition of enzyme activities and the anti-wrinkle effect of polyphenol isolated from the persimmon leaf (Diospyros kaki folium) on human skin. J Dermatol Surg 31:848–854

Arung ET, Matsubara E, Kusuma WI, Sukaton E, Shimizu K, Kondo R (2011) Inhibitory components from the buds of clove (Syzygium aromaticum) on melanin formation in B16 melanoma cells. Fitoterapia 82:198–202

Baek SH, Nam IJ, Kwak HS, Kim KC, Lee SH (2015) Cellular anti-melanogenic effects of a *Euryale ferox* seed extract ethyl acetate fraction via the lysosomal degradation machinery. Int J Mol Sci 16:9217–9235

Batubara I, Darusman LK, Mitsunaga T, Rahminiwati M, Djauhari E (2010) Potency of Indonesian medicinal plants as tyrosinase inhibitor and antioxidant agent. J Biol Sci 2:138–144

Batubara I, Darusman LK, Mitsunaga T, Aoki H, Rahminiwati M, Djauhari E, Yamauchi K (2011) Flavonoid from *Intsia palembanica* as skin whitening agent. J Biol Sci 8:475–480

Briganti S, Camera E, Picardo M (2003) Chemical and instrumental approaches to hyperpigmentation. Pigment Cell Res 16:101–110

Brown LL, Larson SR, Sneden AT (1998) Vanicosides C-F, new phenylpropanoid glycosides from *Polygonum pensylvanicum*. J Nat Prod 61:762–766

Chang TS (2009) An updated review of tyrosinase inhibitors. Intern J Mol Sci 10:2440–2475

Chen WC, Tseng TS, Hsiao NW, Lin YL, Wen ZH, Tsai CC, Lee YC, Lin HH, Tsai KC (2015) Discovery of highly potent tyrosinase inhibitor, T1, with significant anti-melanogenesis ability by zebrafish in vivo assay and computational molecular modeling. Sci Rep 5:7995

Cho JG, Cha BJ, Seo WD, Jeong RH, Shrestha S, Kim JY, Kang HC, Baek NI (2015) Feruloyl sucrose esters from *Oryza sativa* roots and their tyrosinase inhibition activity. Chem Nat Compd 51:1094–1098

Davis AL, Cai Y, Davies AP, Lewis JR (1996) ^{1}H and ^{13}C NMR Assignments of some green tea polyphenols. Magn Reson Chem 34:887–890

Fukuyama Y, Sato T, Miura I, Asakawa Y, Takemoto T (1983) Hydropiperoside, a novel coumaryl glycoside from the root of *Polygonum Hydropiper*. Phytochemistry 22:549–552

Fuyuno I (2004) Spotlight turns on cosmetics for Asian skin. Nature 432:938

Gafner S, Wolfender J-L, Nianga M, Hostettman K (1997) Phenylpropanoid glycosides from *Newbouldia laevis* roots. Phytochemistry 44:687–690

Guria M, Mitra P, Ghosh T, Gupta S, Basu B, Mitra PK (2013) 3, 4 diHydroxyBenzoic acid Isolated from the leaves of Ageratum conyzoides L. Eur J Biotechnol Biosci 1:25–28

Kamatham S, Kumar N, Gudipalli P (2015) Isolation and characterization of gallic acid and methyl gallate from the seed coats of Givotia rottleriformis Griff. and their anti-proliferative effect on human epidermoid carcinoma A431 cells. Toxicol Rep 2:520–529

Kiem PV, Nhiem NX, Cuong NX, Hoa TQ, Huong HT, Huong le M, Minh CV, Kim YH (2008) New phenylpropanoid esters of sucrose from *Polygonum hydropiper* and their antioxidant activity. Arch Pharm Res 31:1477–1482

Kim YJ, Uyama H (2005) Tyrosinase inhibitors from natural and synthetic sources: structure, inhibition mechanism and perspective for the future. CMLS 62:1707–1723

Kobayashi T, Urabe K, Winder A, Jimenez-Cervantes C, Imokawa G, Brewington T, Solano F, Garcia-Borron JC, Hearing VJ (1994) Tyrosinase related protein 1(TRP1) functions as DHICA oxidase in melanin biosynthesis. EMBO J 13:5818–5825

Kuroyanagi M, Fukushima S (1982) Highly oxygenated flavonoids from *Polygonum orientale*. Chem Pharm Bull 30:1163–1168

Le Roy J, Huss B, Creach A, Hawkins S, Neutelings G (2016) Glycosylation is a major regulator of phenylpropanoid availability and biological activity in plants. Front Plant Sci 7:735

Li YJ, He X, Liu LN, Lan YY, Wang AM, Wang YL (2005) Studies on chemical constituents in herb of *Polygonum orientale*. China J Chin Mater Med 30:444–446

Liao SG, Li YT, Zhang LJ, Wang Z, Chen TX, Huang Y, Li J, Wang AM, Li YJ, Lan YY, Wang YL (2013) UPLCPDA-ESI-MS/MS analysis of compounds extracted by cardiac h9c2 cell from *Polygonum orientale*. Phytochem Anal 24:25–35

Medicinal plants of Bangladesh (2018, June 26) Retrieved from: http://www.mpbd.info/plants/persicaria-orientalis.php

Narasimhulu G, Reddy KK, Mohammad J (2014) The genus Polygonum (Polygonaceae): an ethnopaharmacological and phytochemical perspectives - review. Int J Pharm Pharm Sci 6:21–45

Nigam V, Patel A, Malvi R, Gupta B, Vikram P, Goyanar G (2013) Antihyperglycaemic activity on flower of *Polygonum orientale* Linn. using steptozotocin induced diabetic mice model. Int J Res Dev Pharm Life Sci 4:2626–2631

Panda P, Appalashetti M, Judeh ZMA (2011) Phenylpropanoid sucrose esters: plant derived natural products as potential leads for new therapeutics. Curr Med Chem 18:3234–3251

Parvez S, Kang M, Chung HS, Cho C, Hong MC, Shin MK, Bae H (2006) Survey and mechanism of skin depigmenting and lightening agents. Phytother Res 20:921–934

Pawelek JM, Korner AM (1982) The biosynthesis of mammalian melanin. Am Sci 70:136–145

Philippine Medicinal plants (2018, July 18) Retrieved from: http://www.stuartxchange.org/OrientalPepper.html

Salsberg JM, Weinstein M, Shear N, Lee M, Pope E (2016) Impact of Cosmetic Camouflage on the Quality of Life of Children with Skin Disease and Their Families. J Cutan Med Surg 20:211–215

Seidel V, Bailleul F, Libot F, Tillequin F (1997) A phenylpropanoid glycoside from *Ballota nigra*. Phytochemistry 44:691–693

Shajib MS, Datta BK, Sohrab MH, Rashid MA, Nahar L, Sarker SD (2017) Highly Oxygenated Flavonoids from the Leaves of *Nicotiana plumbaginifolia* (Solanaceae). Rec Nat Prod 11:568–572

Shimomura H, Sashida Y, Mimaki Y (1986) Bitter phenylpropanoid glycosides from *Lilium speciosum* var. *rubrum*. Phytochemistry 25:2897–2899

Shin H, Park Y, Jeon YH, Yan XT, Lee KY (2018) Identification of *Polygonum orientale* constituents using high-performance liquid chromatography high-resolution tandem mass spectrometry. Biosci Biotechnol Biochem 82:15–21

Shoyama Y, Hatano K, Nishioka I, Yamagishi T (1987) Phenolic glycosides from *Lilium longiforum*. Phytochemistry 26:2965–2968

Takasaki M, Konoshima T, Kuroki S, Tokuda H, Nishino H (2001) Cancer chemopreventive activity of phenylpropanoid esters of sucrose, vanicoside B and lapathoside A from *Polygonum lapathifolium*. Cancer Lett 200:133–138

Tanimoto S, Tominaga H, Okada Y, Nomura M (2006) Synthesis and cosmetic whitening effect of glycosides derived from several phenylpropanoids. Yakugaku Zasshi 126:173–177

Waterman PG, Mole S (1994) Analysis of phenolic plant metabolites. Blackwell Scientific Publications, Oxford

Wei Y, Chen X, Jiang X, Ma Z, Xiao J (2009) Determination of taxifolin in *Polygonum orientale* and study on its antioxidant activity. J Food Compos Anal 22:154–157

Xiang MX, Xu L, Liu Y, Yan YJ, Hu JY, Su HW (2011) In vitro evaluation of the effects of *Polygonum orientale* L. on proliferation and differentiation of osteoblastic MC3T3-E1 cell. J Med Plants Res 5:231–236

Yamauchi K, Mitsunaga T, Batubara I, Isolation (2011) Identification and tyrosinase inhibitory activities of the extractives from *Allamanda cathartica*. Nat Resour 2:167–172

Yamauchi K, Mitsunaga T, Inagaki M, Suzuki T (2014) Synthesized quercetin derivatives stimulate melanogenesis in B16 melanoma cells by influencing the expression of melanin biosynthesis proteins MITF and p38 MAPK. Bioorg Med Chem 22:3331–3340

Yamauchi K, Mitsunaga T, Itakura Y, Batubara I (2015) Extracellular melanogenesis inhibitory activity and the structure-activity relationships of uginins from Helminthostachys zuylanica roots. J Fitoter 104:69–74

Yokoyama K, Suzuki H, Yasumoto K, Tomita Y, Shibahara S (1994) Molecular cloning and functional analysis of cDNA coding for human DOPAchrome tautomerase/tyrosinase-related protein-2. Biochim Biophys Acta 1217:317–321

Zhao W, Huang XX, Yu LH, Liu QB, Li LZ, Sun Q, Song SJ (2014) Tomensides A-D, new antiproliferative phenylpropanoid sucrose esters from *Prunus tomentosa* leaves. Bioorg & Med Chem Lett 24:2459–2462

Zheng C, Hu C, Ma X, Peng C, Zhang H, Qin L (2012) Cytotoxic phenylpropanoid glycosides from *Fagopyrum tataricum* (L.) Gaertn. Food Chem 132:433–438

Zheng S, Wang D, Meng J, Shen X (1997) Studies on the lignans of *Polygonum orientale*. Acta Bot Sin 40:466–469

Zhong-hua-ben-cao (1999) State administration of traditional Chinese medicine of People's Republic of China. Shanghai Science and Technology Publisher, Shanghai, China.

Zimmermann ML, Sneden AT (1994) Vanicosides A and B, Protein Kinase C Inhibitors from *Polygonum pensylvanicum*. J Nat Prod 57:236–242