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Promotion effect of constituents from the root of *Polygonum multiflorum* on hair growth

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ABSTRACT

Two new compounds, gallic acid ester of torachrysone-8-*O*-β-D-glucoside (**1**) and (*E*)-2,3,5,4′-tetrahydroxystilbene-2-*O*-β-D-xyloside (**4**), along with eight known compounds (**2**, **3**, **5**–**10**) were isolated from a 70% ethanol extract of *Polygonum multiflorum* roots. The structures were determined by ^{1}H and ^{13}C NMR, HMQC, and HMBC spectrometry. Extracts of *P. multiflorum* have been reported to promote hair growth in vivo. This study was carried out to evaluate the effects of isolated compounds from *P. multiflorum* on promoting hair growth using dermal papilla cells (DPCs), which play an important role in hair growth. When DPCs were treated with compounds (**1**–**10**) from *P. multiflorum*, compounds **1**, **2**, **3**, **6**, and **10** increased the proliferation of DPCs compared with the control. Specifically, compound **2** (10 and 20 μM) induced a greater increase in the proliferation of DPCs than minoxidil (10 μM). Additionally, treatment of vibrissa follicles with compound **2** for 21 days increased hair-fiber length significantly. On the basis of this result, further investigation and optimization of these derivatives might help in the development of therapeutic agents for the treatment of alopecia.

Hair loss is an emotionally distressing condition in humans[1] and has shown an increasing trend. Alopecia is known to be caused by disease, nutritional deficiency, aging, and hormone imbalance.[2–5] Although many studies have been done, the underlying mechanisms of hair loss are poorly understood.[5] There have also been many attempts to treat hair loss. Nevertheless, only two drugs (finasteride and minoxidil) have so far been approved for the treatment of hair loss by the US Food and Drug Administration (FDA).[6,7] Finasteride, a 5α-reductase inhibitor, is used to stimulate hair growth in men with androgenic alopecia.[8] Minoxidil, an antihypertensive agent, can promote hair growth by the opening of an ATP-sensitive K^{+}-channel.[9,10] However, the effects of the drugs are limited and transient, with unpredictable efficacy and unwanted side effects.[5,11,12] Thus, more and better new therapeutic measures to prevent hair loss and enhance hair growth are urgently needed.

Hair follicles are composed of several different epithelial cells and dermal papilla cells (DPCs). DPCs are mesenchymally derived fibroblasts located at the base of hair follicles and play an important role in the hair growth. In particular, minoxidil has been reported to have proliferative and anti-apoptotic effects on DPCs.[13] Similarly, Kim et al. 2012[14] reported the promotion effect on hair growth of Acankoreoside J from *Acanthopanax koreanum*. Park et al. 2012[15] studied the hair growth-promoting effect of *Aconiti Ciliare Tuber* extract mediated by the activation of Wnt/β-catenin signaling.

Polygonum multiflorum[16] (Polygonaceae), distributed in northeast Asia, is a well-known traditional Chinese herbal medicine, commonly known as 'Heshouwu' in China.[17] As a tonic, it is often used to prevent premature aging of the kidney and liver, nourishing the blood, fortifying the muscles, tendons and bones, and strengthening and stabilizing the lower back and knees.[18] It has also often been used in Korean traditional medicine because of its anti-allergy, anti-tumor, anti-bacterial, hemostatic, spasmolytic, and analgesic properties.[19] However, it has also been documented that *P. multiflorum* roots possess hair growth activity in traditional medicine, and many studies have shown that it has a strong effect on hair growth and hair color. For example, a recent study demonstrated that an active component, 2,3,5,4′-tetrahydroxystilbene-2-*O*-β-D-glucoside, from *P. multiflorum* induced melanogenesis in melanocytes.[20] Park et al.[21] reported on the hair growth-promoting activities of *P. multiflorum* extracts and the mechanism of action. However, there it is not yet known which compounds contribute to promoting hair growth. And there is no previous report that *P. multiflorum* extract can promote hair growth via proliferation of DPCs.

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Dried roots of *P. multiflorum* were extracted with 70% EtOH three times under refluxing. The 70% EtOH extract was suspended in H_2O and partitioned with CH_2Cl_2 and EtOAc to yield CH_2Cl_2 fraction (A), EtOAc fraction (B), aqueous fraction (C), respectively. The CH_2Cl_2 and EtOAc extracts was subjected to silica gel and reverse-phase (RP) chromatography column to provide compounds **1**–**10**. Compound **1**[22] was obtained as a yellow powder, $[\alpha]_D^{20}$ −72.12 (*c* 0.1 MeOH). It has a molecular formula of $C_{27}H_{28}O_{13}$ as deduced from HR-ESI-MS (*m*/*z* 561.1605 $[M+H]^+$, calcd 561.1603). The ^1H NMR data of **1** revealed the presence of three aromatic protons which were grouped according to their coupling and splitting. The signals at δ_H 7.08 (1H, s, H-4) were divided into an A ring and the signal at δ_H 7.54 (1H, d, *J* = 2.7 Hz, H-7) and 6.89 (1H, d, *J* = 2.7 Hz, H-5) were in a B ring. The coupling constants (2.7 Hz) suggested these two protons should be in a *meta*-position. Two methyl singlets at δ_H 2.74 (3H, s) and 2.46 (3H, s), one methoxyl proton at δ_H 3.87, and an anomeric doublet signal at δ_H 5.61 (1H, d, *J* = 7.4 Hz, H-1′) were also seen in the ^1H NMR spectrum. Using acid hydrolysis, the sugar moiety of **1** was determined to be D-glucose by gas chromatography (GC) analysis.[23] Considering the chemical shifts of the carbon and protons, as well as the coupling constant (7.4 Hz) of the anomeric proton, the sugar was believed to be β-D-glucose. There were two methyl signals at δ_C 20.6 and 32.8, one methoxyl group signal at δ_C 55.8, and one carbonyl signal at δ_C 205.1 (C=O) in the ^{13}C NMR. Ten signals from δ_C 101.9–159.9 indicated the skeleton of this compound should be a naphthalene skeleton. Correlations from the methyl group (δ_H 2.74) to δ_C 124.5 (C-2) and the carbonyl carbon (δ_C 205.1) indicated the substitution of an acetyl group at C-2. ^1H NMR analysis showed an uncoupled aromatic proton (δ_H 7.08) on ring A; the correlation from it to C-4 in HMQC told us this proton should be labeled as C-4 (δ_C 120.0). The other methyl proton at δ_H 2.46, which correlated with C-2 (δ_C 124.5), C-3 (δ_C 135.3), and C-4 (δ_C 120.0) in HMBC suggested the methyl group was attached at C-3. The HMBC correlation from the methoxyl proton (δ_H 3.87) to C-6 (δ_C 159.9) indicated the location of the methoxyl group at C-6. Comparison with reported data suggested the skeleton was a torachrysone.[23] Additionally, the characteristic proton signals at δ_H 7.94 (H-2″, 6″), and carbon signals at δ_C 110.7 (C-2″, 6″), 147.9 (C-3″, 5″), 121.4 (C-1″), 141.4 (C-4″), and 167.7 (C-7″) revealed a galloyl group in compound **1**. The HMBC spectrum (Fig. 2) showed a correlation from the H-1′ (δ_H 5.61) to the carbon signals at C-8 (δ_C 157.1), suggesting that the glucose was located at C-8. The correlations between the H-6′ (δ_H 5.22 and 4.98) and C-7″ (δ_C 167.7), indicating

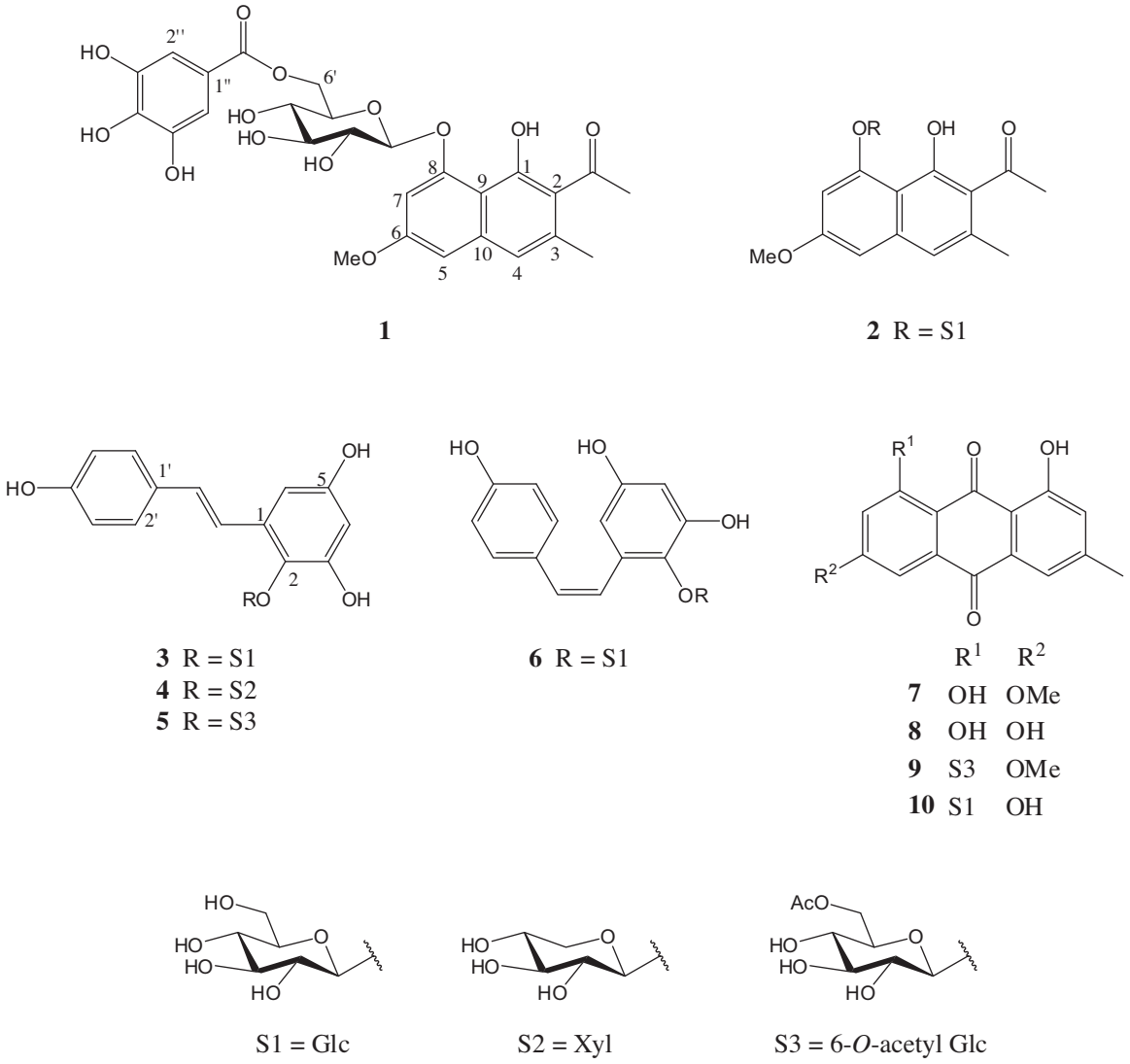

Figure 1. Structure of Compounds (**1**–**10**).

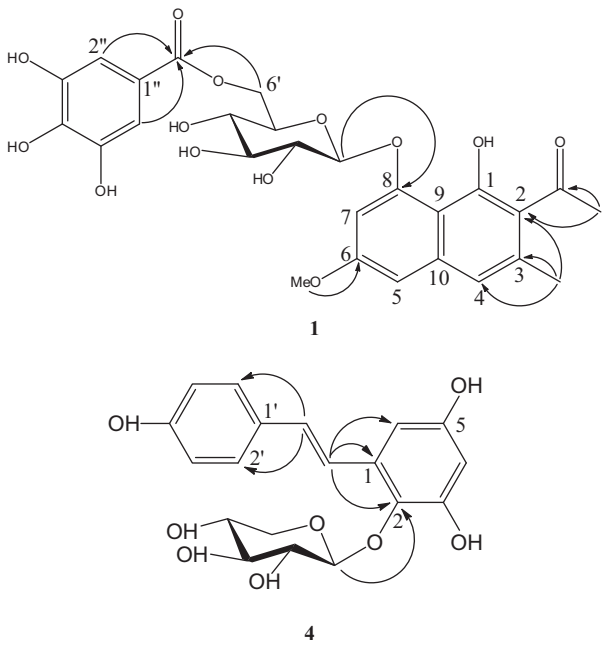

Figure 2. Key HMBC (H→C) correlations of Compounds **1** and **4**.

the presence of the galloyl group attached to C-6′ (δ_C 64.9). In summary, compound **1** was identified as gallic acid ester of torachrysone-8-*O*-β-D-glucoside.

Compound **4**[24] was obtained as a brown powder, $[\alpha]_D^{20}$ +8.68 (*c* 0.1 MeOH); it has a molecular formula of $C_{19}H_{20}O_8$ as deduced from HR-ESI-MS (*m*/*z* 377.1230 $[M+H]^+$, calcd 377.1231). The ^{1}H NMR spectrum showed two aromatic signals at δ_H 7.80 (2H, d, *J* = 8.9 Hz, H-2′ and H-6′) and 7.16 (2H, d, *J* = 8.9 Hz, H-3′ and H-5′), which belong to an A_2B_2-system at A-ring, two doublet signals at δ_H 7.09 (1H, d, *J* = 2.7 Hz, H-4) and δ_H 7.39 (1H, d, *J* = 2.7 Hz, H-6) belong to B-ring, and the coupling constants were 2.7 Hz, suggesting a *meta* position. For the two olefinic proton signals at δ_H 8.53 (1H, d, *J* = 16.2 Hz, H-α) and δ_H 7.42 (1H, d, *J* = 16.2 Hz, H-β), the large coupling constant (16.2 Hz) indicated a *trans*-olefinic coupling pattern. These characteristic signals suggested the skeleton of **4** was stilbene. The ^{1}H NMR spectrum also contained an anomeric proton signal at δ_H 5.19 (1H, d, *J* = 7.6 Hz, H-1″), indicating that compound **4** possessed a monosaccharide moiety. This monosaccharide unit was identified as D-xylose using acid hydrolysis followed by GC analysis.[23] The large coupling constant (7.6 Hz) suggested that sugar was in β form. ^{13}C NMR and DEPT spectra indicated the presence of 19 carbon atoms, and based on the chemical shift indicated the presence of a *trans*-olefinic double bond at δ_C 122.1 (C-α) and 129.9 (C-β), a tetra-substituted aromatic ring at δ_C 133.6 (C-1), 138.3 (C-2), 152.7 (C-3), 104.5 (C-4), 157.1 (C-5), and 103.0 (C-6), and a disubstituted aromatic ring at δ_C 130.1 (C-1′), 129.2 (C-2′), 116.9 (C-3′), 159.3 (C-4′), 116.9 (C-5′), and 129.2 (C-6′). The connectivity of D-xylosyl moiety to C-2 was confirmed by a key correlation between H-1″ (δ_H 5.19) and C-2 (δ_C 138.3) in the HMBC spectrum (Fig. 2). By comparison with reported data, compound **4** was identified as (*E*)-2,3,5,4′-tetrahydroxystilbene-2-*O*-β-D-xyloside.[19]

The other known compounds were identified as torachrysone-8-*O*-β-D-glucoside (**2**),[25] (*E*)-2,3,5,4′-tetrahydroxystilbene-2-*O*-β-D-glucoside (**3**),[19] (*E*)-2,3,5,4′-tetrahydroxystilbene-2-*O*-β-D-(6″-*O*-acetyl)-glucoside (**5**),[26] (*Z*)-2,3,5,4′-tetrahydroxystilbene-2-*O*-β-D-glucoside (**6**),[19] physcion (**7**),[27] emodin (**8**),[28] physcion-8-*O*-β-D-(6′-*O*-acetyl)-glucoside (**9**),[29] and emodin-8-*O*-β-D-glucopyranoside (**10**),[30] (Fig. 1) by comparison of physical and spectroscopic data with literature reports.

Mesenchyme-derived dermal papilla cells (DPC)[31] play an important role in hair growth regulation. Accumulating evidence has shown that the size of the DPCs correlated well with hair growth, and the number of the DPCs was increased in the growing phase of hair cycle.[32,33] Even though *P. multiflorum* extract induces a telogen to anagen transition in the hair cycle in mice,[21] the matter of which compounds can contribute to promoting the hair growth has not been reported. To identify whether compounds isolated from *P. multiflorum* extract could promote hair growth, we examined the proliferative effects of ten different compounds on DPCs. The isolated compounds were identified as torachrysone, stilbenes, and anthraquinone derivatives. Among the tested compounds, compounds **1**, **2**, **3**, **6**, and **10** at 10 μM promoted proliferation of DPCs, by 111.1% (p <0.05), 118.8% (p <0.01), 111.8% (p <0.01), 112.6% (p <0.01) and 113.2% (p <0.01), respectively (Fig. 3). Upon examination of the structure–activity relationship of the isolated compounds (**1**–**10**), the torachrysone derivative (**2**) showed the best effect on proliferation of the DPCs. Comparing

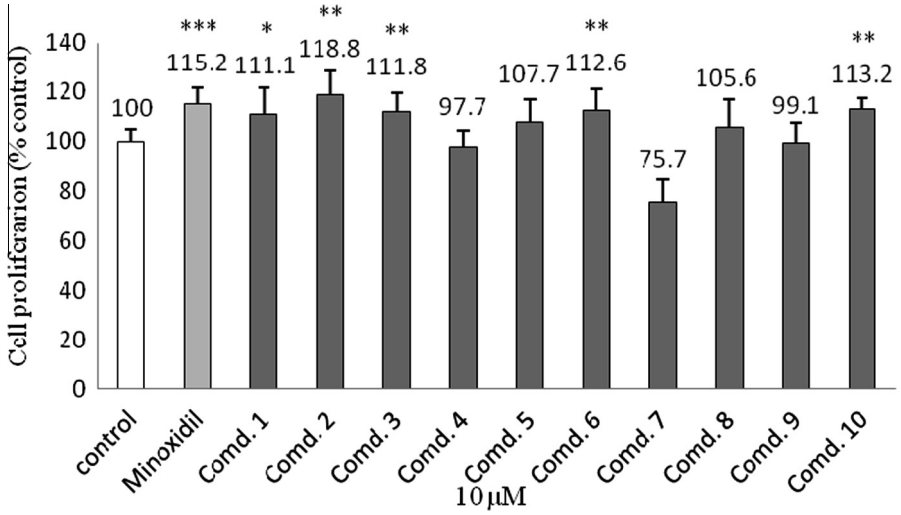

Figure 3. Proliferation effect of the compounds (**1**–**10**) isolated from *P. multiflorum* on DPCs. Immortalized vibrissa DPCs (1.0×10^4 cells/mL) were plated in 96 well plates. DPCs were treated with 10 μM of compounds (**1**–**10**). Cell proliferation was measured using a MTT assay for 4 days. Minoxidil (10 μM) was used as a positive control. Data are presented as the mean ± the S.D. of three independent experiments. $^*p$ <0.05, $^{**}p$ <0.01, $^{***}p$ <0.001 versus control.

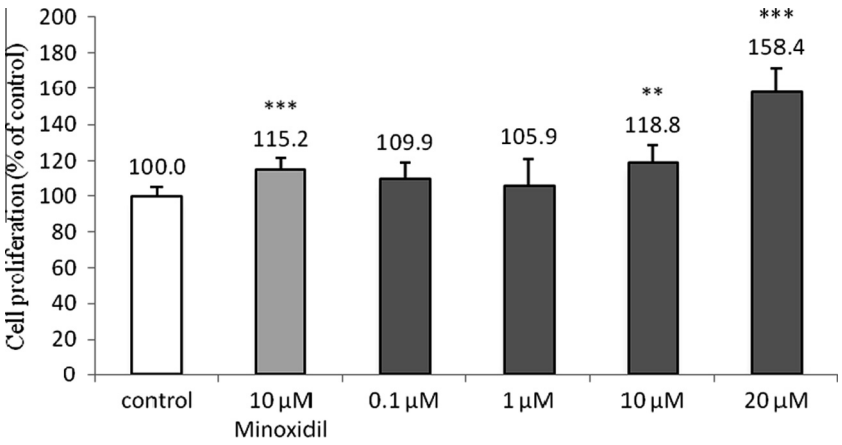

Figure 4. Proliferation effect of compound **2** on DPCs. Immortalized vibrissa DPCs (1.0×10^4 cells/mL) were plated in 96 well plates. DPCs were treated with various concentrations of compound **2**, as indicated. Cell proliferation was measured using a MTT assay for 4 days. Minoxidil (10 μM) was used as a positive control. Data are presented as the mean ± the S.D. of three independent experiments. $^{*}p$ <0.05, $^{**}p$ <0.01, $^{***}p$ <0.001 versus control.

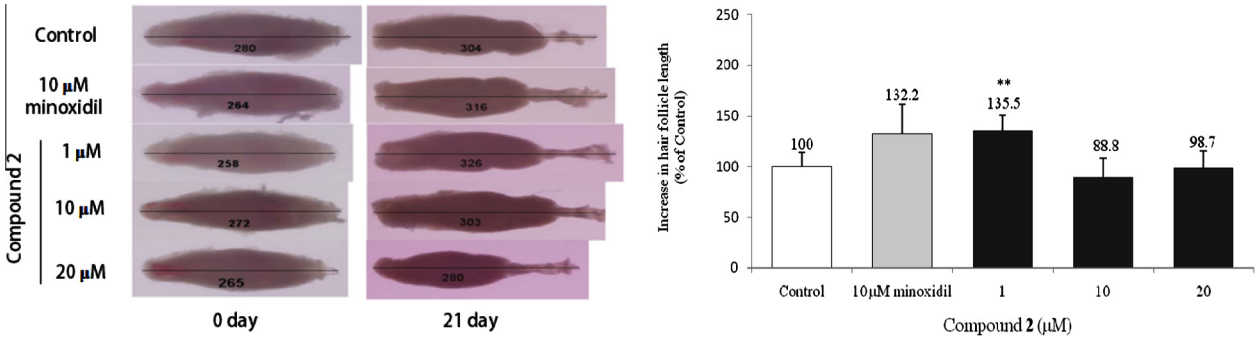

Figure 5. The elongation effect of compound **2** on the hair-fiber length of rat vibrissa follicles. Individual vibrissa follicles from Wistar rats were microdissected and then cultured in William's E medium at 37 °C under 5% CO_2. Vibrissa follicles were then treated with compounds **2** (1, 10 and 20 μM) for 21 days. Stimulation with minoxidil served as a positive control. The difference in the length of vibrissa follicles of the vehicle-treated control group on day 21 was taken to be 100%. Data are presented as the percentage of the length of the treated follicles based on the mean length of the control follicles ± the S.E. $^{**}p$ <0.01 versus control.

with **1**, which linked a galloyl group at C-6 of glucose, suggested the unsubstituted glucose group may play an important role in the proliferative effect on DPCs. Compounds **3**, **6**, and **10** showed strong effects, which also linked to a glucose group of their skeletons. Therefore, the glucose group seems to be a key functional element. In addition, compounds **8** and **10** showed significant effects in the structure– activity relationships of the anthraquinone derivative (**7**–**10**), and these compounds had common structures. The proliferative effect increased significantly when C-6 of skeleton was linked to a hydroxyl group. The other compounds (**7** and **9**), which linked to a mehtoxy group, lacked activity. These results suggested that a hydroxyl group at C-6 of anthraquinone derivative may increase the growth of DPCs. Treatment of DPCs with compound **2** (0.1, 1, 10, 20 μM) resulted in increased proliferation of DPCs by 109.9%, 105.9%, 118.8% (p <0.01), and 158.4% (p <0.001) (Fig. 4). In particular, compound **2** (10 and 20 μM) induced a greater increase in proliferation in DPCs than minoxidil (10 μM). These results suggest that compound **2** may have potential to promote hair growth. We thus further investigated the effects of compound **2** on hair growth using rat vibrissa follicle culture models[34] as a physiologically relevant system for testing hair-growth effects. When rat vibrissa follicles were treated with various concentrations of compound **2** for 21 days, in particular, compound **2** (1 μM) increased the hair-fiber length of vibrissa follicles significantly by 135.5 ± 15.5% (p <0.01) compared with the control group (Fig. 5). As shown in Figures 4 and 5, the dose of compound **2** needed to increase the proliferation of DPCs was higher than that required to increase hair-fiber length. These results suggest the following possibility: in vibrissa follicle culture, whenever the medium was changed every 3 days, compound **2** was also added, with the idea that compound **2** could accumulate in hair follicles. As a result, the concentration of compound **2** sufficient to increase hair-fiber length in the organ culture system could be lower than that needed to increase the proliferation of DPCs.

Interestingly, the results of this study indicate that compounds **1**, **2**, **3**, **6**, and **10** have the potential to promote hair growth through the proliferation of the DPCs. In particular, compound **2** from *P. multiflorum* extract can stimulate hair growth via the promotion of DPC proliferation in hair follicles. The results of this study show that compound **2** and other compounds (torachrysone, stilbenes, and anthraquinone derivatives) with a glucose group from *P. multiflorum* may have therapeutic potential for the promotion of hair growth.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.06.098.

References

1. Cash, T. F. *J. Am. Acad. Dermatol.* **1992**, *26*, 926.
2. Shimomura, Y.; Christiano, A. M. *Annu. Rev. Genomics Hum. Genet.* **2010**, *11*, 109.
3. Daniells, S.; Hardy, G. *Curr. Opin. Clin. Nutr. Metab. Care* **2010**, *13*, 690.
4. Nagase, S.; Kajiura, Y.; Mamada, A.; Abe, H.; Shibuichi, S.; Satoh, N.; Itou, T.; Shinohara, Y.; Amemiya, Y. *J. Cosmet. Sci.* **2009**, *60*, 637.
5. Price, V. H. *N. Engl. J. Med.* **1999**, *341*, 964.
6. Kaufman, K. D.; Olsen, E. A.; Whiting, D.; Savin, R.; DeVillez, R.; Bergfeld, W.; Price, V. H.; van Neste, D.; Roberts, J. L.; Hordinsky, M. *J. Am. Acad. Dermatol.* **1998**, *39*, 578.
7. Burton, J. L.; Marshall, A. *Br. J. Dermatol.* **1979**, *101*, 593.
8. Kaufman, K. D. *Dermatol. Clin.* **1996**, *14*, 697.
9. Hamaoka, H.; Minakuchi, K.; Miyoshi, H.; Arase, S.; Chen, C. H.; Nakaya, Y. *J. Med. Invest.* **1997**, *44*, 73.
10. Shorter, K.; Farjo, N. P.; Picksley, S. M.; Randall, V. A. *FASEB. J.* **2008**, *22*, 1725.
11. Ebner, H.; Müller, E. *Contact Dermatitis* **1995**, *32*, 316.
12. Tu, H. Y.; Zini, A. *Fertil. Steril.* **2011**, *95*, 2125.e13.
13. Han, J. H.; Kwon, O. S.; Chung, J. H.; Cho, K. H.; Eun, H. C.; Kim, K. H. *J. Dermatol. Sci.* **2004**, *34*, 91.
14. Kim, S. C.; Kang, J. I.; Park, D. B.; Lee, Y. K.; Hyun, J. W.; Koh, Y. S.; Yoo, E. S.; Kim, J. A.; Kim, Y. H.; Kang, H. K. *Arch. Pharmacol. Res.* **2012**, *35*, 1495.
15. Park, P. J.; Moon, B. S.; Lee, S. H.; Kim, S. N.; Kim, A. R.; Kim, H. J.; Park, W. S.; Choi, K. Y.; Cho, E. G.; Lee, T. R. *Life Sci.* **2012**, *91*, 935.
16. Sample preparation: root of *Polygonum multiflorum* were purchased from the herbal company, Naemome Dah, Ulsan, Korea in November 2011, and identified by prof. Young Ho Kim, College of Pharmacy, Chungnan National University. A voucher specimen (CNU11103) was deposited at the herbarium of the College of Pharmacy, Chungnan National University in Korea.
17. Chen, L. L.; Huang, X. J.; Li, M. M.; Ou, G. M.; Zhao, B. X.; Chen, M. F.; Zhang, Q. W.; Wang, Y.; Ye, W. C. *Phytochem. Lett.* **2012**, *5*, 756.
18. Yi, T.; Leung, K. S. Y.; Lu, G. H.; Zhang, H.; Chan, K. *Phytochem. Anal.* **2007**, *18*, 181.
19. Kim, H. K.; Choi, Y. H.; Choi, J. S.; Choi, S. U.; Kim, Y. S.; Lee, K. R.; Kim, Y. K.; Ryu, S. Y. *Arch. Pharmacol. Res.* **2008**, *31*, 1225.
20. Jiang, Z.; Xu, J.; Long, M.; Tu, Z.; Yang, G.; He, G. *Life Sci.* **2009**, *85*, 345.
21. Park, H. J.; Zhang, N. N.; Park, D. K. *J. Ethnopharmacol.* **2011**, *135*, 369.
22. *Gallic acid ester of torachrysone-8-O-β-D-glucoside* (**1**)*:* was obtained as a yellow powder; $[\alpha]_D^{20}$ −72.12 (*c* 0.1, MeOH); HR-ESI-MS (*m*/*z* 561.1605 [M+H]$^+$, calcd 561.1603 for $C_{27}H_{29}O_{13}$); ^{1}H NMR (C_5D_5N, 600 MHz) : δ_H 7.94 (2H, s, H-2″, 6″), 7.54 (1H, d, *J* = 2.7 Hz, H-7), 7.08 (1H, s, H-4), 6.89 (1H, d, *J* = 2.7 Hz, H-5), 5.61 (1H, d, *J* = 7.4 Hz, H-1′), 5.22 (1H, d, *J* = 10.1 Hz, H-6′), 4.98 (1H, m, H-6′), 3.87 (3H, s, –OC*H*$_3$), 2.74(3H, s, –C*H*$_3$–C=O), 2.46 (3H, s, –C*H*$_3$); ^{13}C NMR (C_5D_5N, 150 MHz) : δ_C 205.1 (C=O), 167.7 (C-7″), 159.9 (C-6), 157.1 (C-8), 153.4 (C-1), 147.9 (C-3″, 5″), 141.4 (C-4″), 138.5 (C-10), 135.3 (C-3), 124.5 (C-2), 121.4 (C-1″), 120.0 (C-4), 110.7 (C-2″, 6″), 110.4 (C-9), 105.4 (C-7), 105.0 (C-1′), 101.9 (C-5), 78.6 (C-5′), 76.7 (C-3′), 74.9 (C-2′), 71.6 (C-4′), 64.9 (C-6′), 55.8 (–OCH$_3$), 32.8 (–CH$_3$–C=O), 20.6 (–CH$_3$).
23. *Acid hydrolysis:* compounds **1** (5 mg) and **4** (5 mg) were heated in 3 mL 10% HCl (dioxane–H_2O, 1:1) at 90 °C for 3 h. The residue was partitioned between EtOAc and H_2O to give the aglycone and sugar, respectively. The aqueous layer was evaporated using nitrogen until dry to yield a residue and it was dissolved in anhydrous pyridine (200 μL) and then mixed with a pyridine solution of 0.1 M L-cysteine methyl ester hydrochloride (200 μL). After warming to 60 °C for 1 h, trimethylsilylimidazole solution was added, and the reaction solution was warmed at 60 °C for 1 h. The mixture was evaporated in vacuo to yield a dried product, which was partitioned between *n*-hexane and H_2O. The *n*-hexane layer was filtered and analyzed by gas chromatography. Retention times of the monosaccharides derivate as followed D-glucose (tR, 14.11 min) and D-xylose (tR, 12.01 min) were confirmed by comparison with those of authentic standards.
24. *(E)-2,3,5,4′-tetrahydroxystilbene-2-O-β-D-xyloside* (**4**)*:* was obtained as a brown powder, $[\alpha]_D^{20}$ +8.68 (*c* 0.1, MeOH); HR-ESI-MS (*m*/*z* 377.1230 [M+H]$^+$, calcd 377.1231 for $C_{19}H_{21}O_8$); ^{1}H NMR (C_5D_5N, 600 MHz) : δ_H 8.53 (1H, d, *J* = 16.2 Hz, H-α), 7.80 (2H, d, *J* = 8.9 Hz, H-2′, 6′), 7.42 (1H, d, *J* = 16.2 Hz, H-β), 7.39 (1H, d, *J* = 2.7 Hz, H-6), 7.16 (1H, d, *J* = 8.9 Hz, H-3′, 5′), 7.09 (1H, d, *J* = 2.7 Hz, H-4), 5.19 (1H, d, *J* = 7.6 Hz, H-1″), 4.38 (1H, m, H-5″), 4.35 (1H, m, H-2″), 4.25 (1H, m, H-4″), 4.18 (1H, m, H-3″), 3.64 (1H, m, H-5″); ^{13}C NMR (C_5D_5N, 150 MHz): δ_C 159.3 (C-4′), 157.1 (C-5), 152.7 (C-3), 138.3 (C-2), 133.6 (C-1), 130.1 (C-1′), 129.9 (C-β), 129.2 (C-2′, 6′), 122.1 (C-α), 116.9 (C-3′, 5′), 109.5 (C-1″), 104.5 (C-4), 103.0 (C-6), 78.7 (C-3″), 75.8 (C-2″), 71.1 (C-4″), 68.3 (C-5′).
25. Zhang, H.; Guo, Z. J.; Wu, N.; Xu, W. M.; Han, L.; Li, N.; Han, Y. X. *Molecules* **2012**, *17*, 843.
26. Chen, W. S.; Liu, W. Y.; Yang, G. J.; Zhang, W. D.; Chu, Z. Y.; Chen, H. S.; Qiao, C. Z. *Acta Pharmacol. Sin.* **2000**, *35*, 906.
27. Choi, S. G.; Kim, J.; Sung, N. D.; Son, K. H.; Cheon, H. G.; Kim, K. R.; Kwon, B. M. *Nat. Prod. Res.* **2007**, *21*, 487.
28. Kim, Y. M.; Lee, C. H.; Kim, H. G.; Lee, H. S. *J. Agric. Food Chem.* **2004**, *52*, 6096.
29. Qi, H. Y.; Zhang, C. F.; Zhang, M. *Chin. Chem. Lett.* **2005**, *16*, 1050.
30. Kwon, B. M.; Kim, S. H.; Baek, N. I.; Lee, S. I.; Kim, E. J.; Yang, J. H.; Chae, B. S.; Lee, J. H.; Park, H. W.; Park, J. S.; Kim, D. K. *Arch. Pharmacol. Res.* **2009**, *32*, 495.
31. Filsell, W.; Little, J. C.; Stones, A. J.; Granger, S. P.; Bayley, S. A. *J. Cell Sci.* **1994**, *107*, 1761.
32. Jahoda, C. A. B.; Home, K. A.; Oliver, R. F. *Nature* **1984**, *311*, 560.
33. Elliott, K.; Stephenson, T. J.; Messenger, A. G. *J. Invest. Dermatol.* **1999**, *113*, 873.
34. Philpott, M. P.; Green, M. R.; Kealy, T. *Br. J. Dermatol.* **1992**, *127*, 600.