Anti-MMP-2 Activity and Skin-Penetrating Capability of the Chemical Constituents from *Rhodiola rosea*

**Authors**

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**Abstract**

Based on the significant inhibitory activity toward matrix metalloproteinase-2 and collagenase noticed in preliminary studies, crude extracts of *Rhodiola rosea* were partitioned and chromatographed sequentially to afford three new compounds, 1,2,3,6-tetra-o-galloyl-4-O-p-hydroxybenzoyl-β-D-glucopyranoside (E)-creoside I (1), (E)-creoside I (2), and (R,Z)-2-methylhept-2-ene-1,6-diol (3), along with twenty-four known compounds (4–27). Their structures were determined by spectroscopic data analyses. All isolated compounds were subjected to bioactivity assays. In these, 1 specifically inhibited matrix metalloproteinase-2 activity with an IC₅₀ value of 16.3 ± 1.6 µM, while its analogue 1,2,3,6-tetra-o-galloyl-β-D-glucopyranoside (17) inhibited matrix metalloproteinase-2 with an IC₅₀ value of 23.0 ± 4.8 µM. In the collagenase activity assay, the inhibitory effects of 1 and 17 at concentrations of both 20 and 40 µM were more potent than those of the positive control, 1,10-phenanthroline. In order to realize whether 17 could penetrate from the epidermal layer into the basal and dermal layers of the human skin to inhibit the activity of matrix metalloproteinase-2 and collagenase or not, a transdermal penetration test in nude and white mice skins was performed. Penetration percentages of 17 quantified by LC-MS were 27.8% and 74.8% in 24 hours, respectively.

**Supporting information** available online at [http://www.thieme-connect.de/products](http://www.thieme-connect.de/products)

**Key words**

- *Rhodiola rosea*
- Crassulaceae
- matrix metalloproteinase
- collagenase
- 1,2,3,6-tetra-o-galloyl-4-O-p-hydroxybenzoyl-β-D-glucopyranoside
- (E)-creoside I
- (Z)-2-methyl-2-hepten-1,6-diol

**Bibliography**


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**Introduction**

Matrix metalloproteinases (MMPs) are a family of zinc- and calcium-dependent endopeptidases, which are grouped into the metzincin clan of metalloendopeptidases (MPs) together with other families such as ADAMs/adamalysins, astacins, fragilysins, and serralysins. MMPs are found throughout the animal and plant kingdoms, where their distribution is consistent with a Darwinian tree-based pathway [1]. Furthermore, polyplication has led to the presence of several paralogous MMP genes in the same organism: 24 in humans, 26 in sea urchins, 26 in zebrafish, 7 in sea squirts, and 2 in fruit flies. Overexpression of MMPs may cause various inflammatory, malignant, and degenerative symptoms [2]. Among the MMPs studied, gelatinase-A (MMP-2), gelatinase-B (MMP-9), and collagenase-1 (MMP-1) were reported to be responsible for the signal transduction of dermal photoaging [3], and inhibition of the activities of these two enzymes could potentially slow down skin aging. Thus, the search for bioactive compounds that can regulate the activities of MMP-1, -2, and -9 from natural resources is one of the key steps in delaying skin aging.

*Rhodiola rosea* L. (Crassulaceae), an herbaceous plant, is used in Asian and Eastern European traditional medicines for its pressure-reducing [4], neurosystem-stimulating [5], fatigue-removing [6], hypobaropathy-preventing [7], antidepressive [7], anticancer [8], antiaging [9], cardiovascular-protective [10], and hepatoprotective effects [11]. Chemical investigations on this plant have revealed many chemical entities, including phenolic acids [12], phenolethanoids [13], phenylpropenoids [14], flavonoids [15], monoterpenes [4], and glycosides [16]. In a preliminary biological evaluation, crude extracts of *R. rosea* roots exhibited inhibitory activities toward MMP-2 and collagenase at a concentration of 100 µg/mL [17]. An investigation of the active principles of this plant was thus undertaken by using a bioassay-guided method. Of the tested water, n-butanol, and ethyl acetate layers, the ethyl acetate layer

was the most potent. The subsequent isolation and identification of bioactive components was focused on this layer and led to the isolation and characterization of three new compounds (1–3; Fig. 1) and twenty-four known compounds (4–27). The isolation and structural elucidation of the previously unreported compounds are described in this paper along with their bioactivities.

Results and Discussion

An ethanolic extract of the roots of *R. rosea* was partitioned in a preliminary manner to give an ethyl acetate soluble layer. Flash column separation of this layer over silica gel afforded three previously unreported chemical entities (five known compounds were identified to be methyl 4-hydroxybenzoate (4) [18], 3-methyl-but-2-en-1-yl-β-D-glucopyranoside (5) [17], (Z)-2-methyl-6-oxo-2-hepten-1-ol (6) [20], gallic acid (7) [21], 4-hydroxyphenyl-2-ethyl-β-D-glucopyranoside (8) [22], tyrosol (9) [23], methyl gallate (10) [24], p-coumaric acid (11) [25], caffeic acid (12) [25], aspergillol B (13) [26], 2,3-dihydrokaempferol (14) [27]. (E)-2-methyl-6-oxo-2-hepten-1-ol (15) [17], 1,2,6-tri-O-galloyl-β-D-glucopyranoside (16) [28], 1,2,3,6-tetra-O-galloyl-β-D-glucopyranoside (17) [28], p-hydroxyphenyl-nethyl alcohol 1-O-D-(6′-O-galloyl)-glucopyranoside (18) [29], herbacetin 7-O-α-L-rhamnopyranoside (19) [30], kaempferol 3-O-α-L-rhamnopyranoside (20) [30], epicatechin-3-O-gallate (21) [31], 1,1-dimethylprop-2-en-1-yl-β-D-glucopyranoside (22) [20], 3-methyl-but-2-en-1-yl-β-D-glucopyranoside (23) [32], (Z)-creoside I (24) [33], creoside III (25) [33], salidroside (26) [34], and icariside D2 (27) [35] based on spectroscopic data analysis and comparison to the literature.

Compound 1, obtained as amorphous white powder, had a formula of C14H24O7, as determined using 13 C NMR (Table 1), as well as a pseudo-molecular ion [M + H]+ at m/z 907.1241 in negative ion high-resolution electrospray ionization mass spectrometry (HR-ESIMS). IR absorption bands of 1 showed a conjugated ester carbonyl (1702 cm−1) along with aromatic functionalities (1609 and 1536 cm−1). In the 1 H NMR spectrum of 1 (Table 1), signals at δH 6.24 (1 H, d, J = 8.3 Hz, H-1), 5.58 (1 H, d, J = 8.3, 9.6 Hz, H-2), 5.91 (1 H, t, J = 9.6 Hz, H-3), 5.63 (1 H, t, J = 9.6 Hz, H-4), 4.40 (1 H, H-5), 4.41 (1 H, H-6a), 4.51 (1 H, d, J = 10.3 Hz, H-6b), 7.05 (2 H, s, H-2′, -6′), 6.95 (2 H, s, H-2′′, -6′′), 6.80 (2 H, s, H-2′′′, -6′′′), and 7.10 (2 H, s, H-3′′′′, -6′′′′) were attributable to a β-D-glucopyranoside bearing four galloyl groups at C-1, -2, -3, and C-6 via ester linkages; this conclusion was corroborated by the large mutual coupled J values of H-1–H-5 and heteronuclear long-range correlations in the heteronuclear multiple-bond correlation (HMBC) experiment (Fig. 2A). Two additional mutual coupled resonances at δH 6.75 (2 H, d, J = 8.7 Hz) and 7.78 (2 H, d, J = 8.7 Hz) were characteristic signals for p-hydroxybenzoyl located at CO-4 as evidenced by a cross-peak of H-4/7 in the HMBC spectrum. Accordingly, 1 was determined as shown and named 1,2,3,6-tetra-O-galloyl-4-p-hydroxybenzoyl-β-D-glucopyranoside.

Compound 2 was isolated as amorphous white powder with the molecular formula C14H24O7Na, as determined by positive ion HR-ESIMS, and showed an [M + Na]+ ion at m/z 327.1428 (calcd. for C14H24O7Na, 327.1420). Conspicuous absorptions at 3389 and 1689 cm−1 in the IR spectrum of 2 indicated the presence of hydroxyl and ketone functionalities, respectively. The 1H NMR (Table 2) spectrum coupled with the correlation spectroscopy (COSY) spectrum of 2 showed signals at δH 4.22 (1 H, d, J = 8.0 Hz, H-1′), 3.18 (1 H, dd, J = 8.0, 8.5 Hz, H-2′), 3.33 (1 H, t, J = 8.5 Hz, H-3′), 3.27 (1 H, t, J = 8.5 Hz, H-4′), 3.22 (1 H, m, H-5′), 3.65 (1 H, dd, J = 5.5, 11.5 Hz, H-6′a), and 3.85 (1 H, dd, J = 2.0, 11.5 Hz, H-6′b), indicating a β-D-glucopyranoside moiety. Further, the 1H, 13 C NMR, COSY, and heteronuclear multiple-quanton correlation (HMBC) spectra of 2 indicated the presence of

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Table 1 13 C (125 MHz) and 1 H NMR (500 MHz) data for compound 1 (in CD3OD).

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*Signals without multiplicity were overlapped and picked up from the HSQC spectrum.*

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two methyls $[^{1}H] 2.12$ (3 H, s, H3–7) and 1.69 (3 H, s, H3–8) in the residual aglycone part, three methylenes $[^{1}H] 4.02$ and 4.18 (each 1 H, d, $J = 11.5$ Hz, H2–1), vicinal coupling $[2.28 (2 H, m, H2–4) and 2.53 (2 H, t, J = 7.0$ Hz, H2–5)], and an olefinic methine $[^{1}H] 5.42$ (1 H, t, $J = 6.5$ Hz, H–3). In the HMBC spectrum of 2 (© Fig. 2B), cross-peaks of $[^{1}H] 1.69$ (H3–8)/$[^{13}C] 75.6$, $[^{13}C] 133.9$ (C–2) and $[^{13}C] 128.3$ (C–3), $[^{1}H] 5.42$ (H–3)/$[^{13}C] 23.0$ (C–4) and 43.7 (C–5), $[^{1}H] 2.12$ (H3–7)/$[^{13}C] 211.4$ (C–6) and 43.7 (C–5) and $[^{1}H] 4.22$ (H–1)/$[^{13}C] 75.6$ (C–1) indicated that the aglycone of 2 was connected with the C–1′ of β-D-glucopyranoside via an ether linkage to form creoside I. The configuration of $[^{1}H] 2$ was determined to be in E form owing to the $[^{13}C] 14.1$ of C–8 in 2 in contrast with $[^{13}C] 21.9$ of C–8 in (Z)-creoside I (25). Consequently, the structure of 2 was elucidated as shown and the compound was named (E)-creoside I.

Compound 3 was obtained as oil with an optical rotation $[\alpha]^{25}_{D} = -42.2^\circ$ (c 0.23, MeOH). Comparison of the $[^{1}H]$ and $[^{13}C]$ NMR spectra of 3 and 2 revealed the major distinctive differences to be primary alcohol ($[^{13}C] 60.0$, C–1), a secondary hydroxyl group ($[^{13}C] 66.5$, C–6), and Z form $[^{1}H]$ (20.2, C–8) in 3 as opposed to the ether group ($[^{13}C] 75.6$, C–1), ketone group ($[^{13}C] 211.4$, C–6), and E form $[^{1}H]$ (14.1, C–8) in 2, respectively. The configuration of the $[^{1}H]$ in 3 was also verified by a nuclear Overhauser effect (NOE) enhancement signal of H–3 at $[^{1}H] 1.75$ after radiation of H–3 at $[^{1}H] 5.27$, which indicated a Z form double bond. The chirality of C–6 was determined to be R by comparing the optical rotation of 3, $[\alpha]^{25}_{D} = -42.2^\circ$, with that of (R)-6-methylhept-5-en-2-ol, $[\alpha]^{25}_{D} = -14.5^\circ$ [36]. Thus, compound 3 was assigned as shown and named (RZ)-2-methylhept-2-ene-1,6-diol.

In the MMP inhibitory activity assay, 1,2,3,6-tetra-O-galloyl-4-O-p-hydroxybenzoyl-β-D-glucopyranoside (1) inhibited MMP-2 activity with an IC$_{50}$ of 16.3 ± 1.6 µM, while its analog 1,2,3,6-tetra-O-galloyl-β-D-glucopyranoside (25) inhibited MMP-2 with an IC$_{50}$ value of 23.0 ± 4.8 µM. In the collagenase inhibitory activity assay, the effects of 1 and 17 at concentrations of both 20 and 40 µM were significant and more potent than those of the positive control, 1,10-phenanthroline (© Table 3). When a concentration of 20 µM of compound 17 was applied, an inhibitory activity of over 50% could be achieved. Owing to its promising activity in the enzymatic assay and in an attempt to realize whether 17 could penetrate from the epidermal layer into the basal and dermal layers of human skin or not, a transdermal penetration assay of 20 µM of compound 17 was performed using nude and white mouse skins. The nude mouse skin with sparse hair follicles mimicked human skin, and the white mouse skin with compact hair follicles was used for comparison. The transdermal penetration percentages of 17 in the nude mouse skin and the white mouse skin were 27.8% and 74.8% (© Table 4), respectively, in 24 h as determined by using LC-MS. In the same conditions, the transdermal penetration percentage of genistein, a positive control used in this study, was 3.7%. Compound 17 was thus speculated to be able to pene-

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**Table 3** $[^{13}C]$ (125 MHz) and $[^{1}H]$ NMR (500 MHz) data for compounds 2 and 3 (in CD$_3$OD).

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<td>3.65 (1 H, d, $J = 5.5$, 11.5 Hz)</td>
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turate from the epidermal layer into the basal and dermal layers of human skin to inhibit the activity of MMP-2 and collagenase, and to mitigate skin aging.

**Materials and Methods**

**General experimental procedures**

Optical rotations were measured on a JASCO P-1020 polarimeter. ¹H and ¹³C NMR were performed with a Bruker Avance DRX-500 spectrometer. Mass spectra were obtained using an API 4000 Q-TRAP ESI spectrometer. Optical rotations were measured on a JASCO P-1020 polarimeter.

**Chemicals and reagents**

HPLC grade solvents, n-hexane, ethyl acetate, methanol, and acetonitrile were purchased from J.T. Baker. Epigallocatechin gallate, genistein, and 1,10-phenanthroline (all purities > 95%) were purchased from Sigma-Aldrich.

**Plant materials**

Dried roots of *R. rosea* were purchased from Scientific Pharmaceutical Elite Company (Batch No. 106), Taipei, Taiwan, on September 8, 2009. A voucher specimen (No. TMU090908) was identified by Hsiu-Wen Huang, Taiwan Endemic Species Research Institute, ChiChi, Taiwan, and deposited at the Institute of Pharmacognosy, Taipei Medical University, Taipei, Taiwan.

**Extraction and isolation**

Dried roots (10.0 kg) of *R. rosea* were smashed and extracted three times with 40 L of ethanol, which was filtered and rotary evaporated to give a black residue (1758 g). This residue was then suspended in H₂O (3 L) and partitioned with an equal volume of ethyl acetate three times. The ethyl acetate layer was evaporated to dryness under vacuum (415 g). The residue, and spots were detected by spraying with vanillin-sulfuric acid followed by heating. Then, all fractions were combined into six portions (I–VI) according to the results of the TLC analyses; they were then redissolved in a minimum volume of the n-hex/ethyl acetate mixtures for subsequent HPLC analysis. Portion II eluted by n-hexane/ethyl acetate (95:5) was purified by performing semipreparative HPLC (Hibar® Fertigsäule, 10 × 250 mm) using n-hexane/ethyl acetate (96:4) as the eluent at a flow rate of 3 mL/min to afford 6 (4.2 mg, tR = 19.8 min), 3 (6.1 mg, tR = 21.2 min), 5 (32 mg, tR = 23.5 min), and 7 (79 mg, tR = 28.0 min). The same portion was purified by performing semipreparative HPLC (Phenomenex® Luna, 10 × 250 mm) using n-hexane/ethyl acetate (99:1) as the eluent at a flow rate of 3 mL/min to afford 4 (36 mg, tR = 32.5 min). Portion III, eluted by n-hexane/ethyl acetate (90:10), was purified by performing semipreparative HPLC (Phenomenex® Luna, 10 × 250 mm) using n-hexane/acetone (99:1) as the eluent at a flow rate of 3 mL/min to afford 9 (25 mg, tR = 25.6 min). The same portion was purified by performing semipreparative HPLC (Phenomenex® Luna, 10 × 250 mm) using n-hexane/ethyl acetate (85:15) as the eluent at a flow rate of 3 mL/min to afford 10 (16 mg, tR = 27.6 min), 11 (24 mg, tR = 32.5 min), and 12 (24 mg, tR = 42.5 min). The same portion was purified by performing semipreparative HPLC (Phenomenex® Luna, 10 × 250 mm) using n-hexane/ethyl acetate (78:22) as the eluent at a flow rate of 3 mL/min to obtain 14 (30 mg, tR = 21.0 min) and 13 (22 mg, tR = 24.6 min). The same portion was purified by performing semipreparative HPLC (Phenomenex® Luna, 10 × 250 mm) using n-hexane/ethyl acetate (85:15) as the eluent at a flow rate of 3 mL/min to obtain 19 (25 mg, fR = 13.2 min), 17 (55 mg, fR = 16.3 min), 16 (132 mg, tR = 21.8 min), and 18 (39 mg, tR = 25.6 min). Portion V, eluted by n-hexane/ethyl acetate (60:40), was purified by performing semipreparative HPLC (Hibar® Fertigsäule, 10 × 250 mm) using n-hexane/ethyl acetate (68:27:5) as the eluent at a flow rate of 3 mL/min to afford 20 (5.3 mg, tR = 10.2 min), 22 (63 mg, tR = 20.0 min), 21 (84 mg, tR = 22.0 min), and 23 (33 mg, tR = 26.5 min). The same portion was purified by performing semipreparative HPLC (Hibar® Fertigsäule, 10 × 250 mm) using n-hexane/ethyl acetate (72:28) as the eluent at a flow rate of 3 mL/min to afford 2 (24 mg, tR = 24.3 min), 24 (4.6 mg, tR = 32.3 min), and 25 (33 mg, tR = 38.7 min). Portion VI, eluted by n-hexane/ethyl acetate (40:60), was purified by performing semipreparative HPLC (Hibar® Fertigsäule, 10 × 250 mm) using n-hexane/ethyl acetate (60:40).
Identification of isolated compounds

All the isolated compounds were identified by interpreting their 
$^1$H, $^{13}$C, and 2D NMR spectra, including COSY, NOESY, HMOC, and 
HMBC spectra, which were further supported by IR, LR-, HR-MS, 
and optical rotation data.

$1,2,3,6$-Tetra-O-galloyl-4-O-p-hydroxybenzoyl-$
\beta$-D-glucopyranoside (1): Amorphous white powder; [α]$_D^{25}$ + 38.75 (c 0.08, CH$_2$OH); 
IR (neat) $\nu_{max}$ 3375, 1702, 1609, 1536; negative HRESIMS $m/z$ 907.1241 [M + H]$^+$ (calcld. for C$_{49}$H$_{51}$O$_{24}$, 907.1205); for $^1$H and 
$^{13}$C NMR data see Table 1.

(E)-Cresidine I (2): Amorphous white powder; [α]$_D^{25}$ − 58.0 (c 0.05, 
CH$_2$OH); IR (neat) $\nu_{max}$ 3389, 1698; ESIMS $m/z$ 327.2 [M + Na]$^+$;
for $^1$H and $^{13}$C NMR data see Table 2.

(R,Z)-2-Methylhept-2-ene-1,6-diol (3): Colorless oil; [α]$_D^{25}$ − 42.2 (c 
0.23, CH$_2$OH); ESIMS $m/z$ 166.2 [M + Na]$^+$; for $^1$H and $^{13}$C NMR data see 
Table 2.

Matrix metalloproteinase-2 inhibitory activity assay

To evaluate the MMP-2 inhibitory activity of the galloyl derivatives 
1, 7, and 16-19, gelatin zymography was conducted [37].
Briefly, HT1080 cell suspension (5 × 10$^5$ cells/mL) was placed in 
24-well cell culture plates for 24 h of incubation at 37 °C. Subse-
sequently, the cells were treated with the vehicle (DMSO), com-
ponents. The PAGE gel contained 1% gel-

Collagenase inhibitory activity assay

DQ™ (EnzChek Gelatinase/Collagenase Assay Kit, E-12055) is a 
type of fluorescent material that can bond with gelatin to form 
DQ-gelatin. Since the capability of collagenase type IV to induce 
gelatin hydrolysis has been demonstrated, DQ-gelatin can be 
a substitute for collagenase. Thus, the principle of this experiment 
was to measure the activity of collagenase by determining the 
emission intensity of DQ fluorescence because the chemical bond 
between DQ-gelatin could be enzymatically digested by collage-
nase. The fluorescence intensity can be measured using a fluores-
cence microplate reader equipped with standard fluorescence fil-
ters. The fluorescence absorption and emission wavelengths for
the digestion product from DQ-gelatin and the DQ collagen sub-
strate were 495 nm and 515 nm, respectively. Therefore, a fluo-
rescence emission intensity higher than that of the blank at a 
wavelength of 515 nm is an indicator of collagenase activity. 
In other words, the resulting fluorescence emission intensity would 
decrease at 515 nm if the collagenase inhibitors, compounds 1 
and 17, were applied. The results of the collagenase assay are pre-
sent as mean ± SD values.

Transdermal penetration test

To evaluate the effectiveness of a topical agent of compound 17 
within a larger amount, a transdermal penetration test was per-
formed. The apparatus used for this experiment was a Franz-type 
diffusion cell, consisting of a set of vertical double-diffusion and 
detachable glass containers. The upper donor chamber of the cell 
is a hollow cylinder for sample injection, the bottom contact sur-
fase of the donor chamber is provided for close integration. The 
lower receptor chamber is a double-layered hollow cylindrical 
diffusion container, of which the inner layer is made of glass, con-
taining the fluid for sampling; the outer layer of the receptor 
chamber is filled with circulating water, maintained at 37 ± 1 °C 
to simulate the human body. The contact area between the donor 
chamber and the receptor chamber was 0.785 cm$^2$ (the actual 
penetration area), and nude or white mouse (n = 3) skin (epider-
mis facing upwards) was fixed using metal clips to serve as the in 
vitro transdermal penetration barrier. The experimental method 
is presented below. First, compound 17 was dissolved in 30 wt% 
propylene glycol aqueous solution, followed by a transfer of 
0.5 mL of aqueous solution (containing 0.25 mg) of compound 17 
into the donor chamber of the Franz-type diffusion cell. The top 
of the open-ended donor chamber was covered tightly with Par-
afilmin, and the inner receptor chamber was filled with 5.5 mL of 
the buffer solution (30 wt% ethanol and phosphate buffer solu-
tion; pH 7.4), under continuously stirring at 600 rpm. The buffer 
solution was sampled (0.3 mL) at time points of 1, 2, 4, 6, 8, 10, 12, 
24, 36, and 48 h, and was replaced with an equal volume (0.3 mL) 
of the buffer solution to maintain the same volume of fluid in the 
diffusion cell. Finally, the accumulated compound 17 in the solu-
tion in the receptor chamber was analyzed by liquid chromatogra-
phy-tandem mass spectrometry (LC-MS/MS), and the results 
represented the amount of compound 17 that could penetrate 
through the skin and reach the blood vessels. After completing 
this experimental step, deionized water was used to wash com-
pound 17 off the nude and white mouse skin surfaces. Thereafter, 
the skins were taken out of the apparatus and trimmed to obtain a 
circle with a diameter equal to that of the donor chamber by using 
surgical scissors, and the weight of the sample was recorded. The 
skin samples were then homogenized with 1 mL of alcohol at 
300 rpm for 5 min, followed by centrifugation at 10000 rpm for 
5 min. The supernatant was filtered using 0.45 µm PVDF and the 
amount of compound 17 in the skin was quantified by perform-
ing LC-MS. Genistein was used as a positive control following the 
same method.

Transdermal penetration analysis using high-performance liquid chromatography tandem mass spectrometry

The HPLC-MS/MS apparatus used consisted of an Agilent 1100 
HPLC system coupled to an Applied Biosystems 4000 triple quad-
rupole mass spectrometer with an electrospray ionization source 
for the simultaneous detection of 1,2,3,6-tetra-O-galloyl-$
\beta$-D-glucopyranoside (17) and genistein. The chromatographic sepa-
rati on was performed on a C18 Phenomenex column (100 × 4.6 mm, 5 µm). The injection volume was 10 µL. The flow rate was set to 0.5 mL/min and the gradient used was as follows (where A = water and B = acetonitrile): t = 0–1.2 min, A: B (80: 10 v/v); t = 1.2–2.5 min, A: B (80: 10 v/v); t = 2.5–3.5 min, A: B (80: 10 v/v) to A: B (10: 80 v/v); t = 3.5–6.5 min, A: B (80: 10 v/v). Mass detection and quantification were performed in the negative mode using multiple reaction monitoring (MRM) of the precursor/product ion pair at m/z 787/617 and 269/133 for compound 17 and genistein, respectively. The optimized parameters for mass detection were as follows: curtain gas, 10 psi; nebulizer gas (Gas 1), 60 psi; auxiliary gas (Gas 2), 65 psi; and turbo ion spray temperature, 425 °C. Data acquisition and processing were performed using Analyst 1.4 software (AB SCIEX).

Statistical analysis

The experimental results are expressed as mean ± standard error. Data were assessed using the Student-Newman-Keuls test and p < 0.05, p < 0.01, and p < 0.001 was considered significant.

Supporting information

Spectral data of compounds 1–3 are available as Supporting Information.

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Conflict of Interest

The authors declare no conflict of interest.

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