

## Anti-inflammatory activities of fractions from *Geranium nepalense* and related polyphenols

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**ABSTRACT:** *Geranium nepalense* Sweet is a common Chinese herbal medicine and has been used as influenza, dysentery, antiphlogistic and analgesic tonic, hemostatic, stomachic, and antidiabetic drugs. The anti-inflammatory effects of *G. nepalense* on tetradecanoyl phorbol acetate (TPA)-induced mouse ear edema were studied in this work. The results showed that ethyl acetate fraction of the water extract of *G. nepalense* possessed significant activity at 2.5 g/kg ( $p < 0.01$ ) with aspirin as a positive control (0.6 g/kg). Six polyphenolic compounds, including three flavonoids, *i.e.* kaempferol, kaempferol-7-*O*- $\beta$ -D-glucopyranoside, and quercetin-7-*O*- $\alpha$ -rhamnopyranoside, and two tannins, *i.e.* pyrogallol and gallic acid, and one lignin, *i.e.* epipinoresinol, were isolated and characterized from ethyl acetate fraction. The isolation of polyphenols provides a clue for beneficial effects of *G. nepalense* in the demonstrated anti-inflammatory activity.

**Keywords:** *Geranium nepalense*, anti-inflammatory activity, TPA-induced mouse ear edema, polyphenolic, flavonoids

### 1. Introduction

*Geranium nepalense* Sweet (Geraniaceae) is widely distributed in China (1). It has been used to treat various inflammatory conditions, including influenza, dysentery, antiphlogistic and analgesic, and used as a Chinese herbal medicine (2,3). The extract of *G. nepalense* inhibited tetradecanoyl phorbol acetate (TPA)-induced edema in mouse ears in our screening

for anti-inflammatory components. Here, we report the anti-inflammatory activities of extract fractions and the compounds isolated from the active extract of *G. nepalense*.

### 2. Materials and Methods

#### 2.1. General experimental procedures

The NMR spectra were obtained on a Bruker AM-400 spectrometer operating at 400 MHz for <sup>1</sup>H-NMR and 100 MHz for <sup>13</sup>C-NMR, respectively. The spectra of electro spray ionization-mass spectrometry (ESI-MS) were recorded on a Finnigan LCQ Advantage Max ion trap mass spectrometer (Thermo Finnigan, USA). The isolation process was conducted on silica gel (200-300 meshes, Qingdao Marine Chemical, China), Sephadex LH-20 (25-100  $\mu$ m, Fluka, Switzerland). Thin layer chromatography (TLC) was carried out on silica gel GF254 plates (0.2 mm thickness, 5  $\times$  10 cm, Qingdao Marine Chemical, China).

#### 2.2. Plant material

*G. nepalense* Sweet was collected in Songhuaba, Kunming, Yunnan, China. The authentication process was carried out by Dr. Jianying Xiang (Kunming Institute of Botany, Chinese Academic of Sciences). A voucher specimen was deposited in the Kunming Institute of Botany, Chinese Academic of Sciences (Kunming, Yunnan, China).

#### 2.3. Animals

The Kunming mice were purchased from Yunnan Baiyao Group Company Limited (Kunming, Yunnan, China). Animal Ethics Committee (AEC) approvals were obtained for the experimental protocols. The AEC oversees animal programs, facilities and procedures. Mice were housed in a climate-controlled environment with a 12 h light/dark cycle and were provided with free access to food and water during the experiment.

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#### 2.4. Extraction and fractionation

The dried and cut material (2.0 kg) was soaked in distilled water and boiled for three times. The water solution was combined and concentrated *in vacuo* to about 500 mL. The concentrated water solution was then partitioned with ethyl acetate and *n*-butanol successively. After reevaporation under reduced pressure, ethyl acetate fraction (37 g) (marked as GN-EA) and *n*-butanol fraction (33 g) (marked as GN-BU) were obtained respectively. Finally, the left water fraction was concentrated to dry and marked as GN-W.

#### 2.5. Anti-inflammatory activities of extract fractions of *G. nepalense*

The anti-inflammatory activities of GN-EA, GN-Bu, and GN-W were evaluated in a TPA-induced mouse ear edema model. The inflammation model was established according to Hu's and Agut's method (4,5).

A total of 75 Kunming mice were allotted to five groups of 15 each in a completely randomized design. The mice of each group were treated by gastric perfusion of none, 0.6 g/kg aspirin, 2.5 g/kg GN-EA, 2.5 g/kg GN-Bu, and 7.5 g/kg GN-W 30 min prior to each TPA (0.05 mL) treatment once a day for 3 days.

The mice were sacrificed 1 h after the last TPA treatment. Ear punches (7 mm diameter) were taken from each group and weighted. The *t* test with different samples was adopted for comparison between groups.

#### 2.6. Isolation of GN-EA fraction

The ethyl acetate fraction (32 g) was subjected to column chromatography (CC) over silica gel eluted with a solvent system of CHCl<sub>3</sub>/MeOH in gradient (100:1; 100:5; 100:10, and MeOH) to obtain 9 subfractions (Fr. 1-9) based on the TLC analysis. Fr. 4 (606 mg) was subjected to CC over silica gel (15 g) and eluted with petroleum ether/acetone (10:1; 5:1; 3:1) to obtain Fr. 4a (25 mg) and further purified by CC over Sephadex LH-20 eluted with MeOH to yield **1** (8 mg). Fr. 8 was purified by repeated CC over Sephadex LH-20 eluted with MeOH to yield **2** (90.5 mg) and **3** (24.0 mg). Fr. 5 (900 mg) was subjected to CC over Sephadex LH-20 eluted with MeOH to obtain **4** (670 mg). Fr. 6 was purified by CC over Sephadex LH-20 eluted with MeOH to yield **5** (700 mg). Fr. 1 (400 mg) was subjected to CC over silica gel (15 g) and eluted with petroleum ether/acetone (10:1; 5:1) to obtain Fr. 1a (25 mg). Fr. 1a was further purified by CC over Sephadex LH-20 eluted with MeOH to yield **6** (6.5 mg).

### 3. Results and Discussion

#### 3.1. Anti-inflammatory activities of *G. nepalense* fractions

Preliminary phytochemical screening and evaluation of

anti-inflammatory components indicated that organic acids, flavonoids, polyphenolic, tannin, and essential oil may be responsible, at least in part, for the anti-inflammatory effects of the total extract of *Geranium* (3,6). Therefore, the TPA-induced ear edema model was employed with the objective of seeking the major bioactive fraction from *G. nepalense*. Both GN-EA and GN-Bu fractions exhibited significant ( $p < 0.01$ ) anti-inflammatory activities on TPA-induced ear edema model at 2.5 g/kg in our study (Table 1).

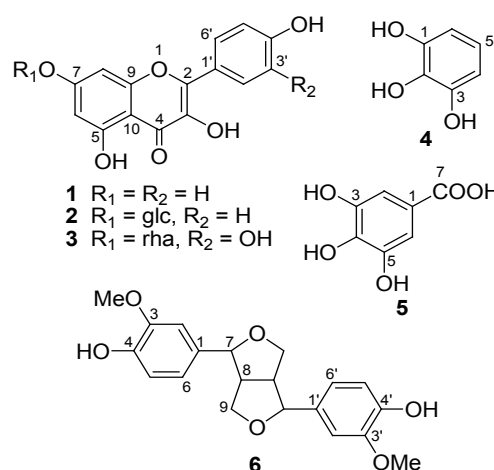
#### 3.2. Chemical structures of the isolated compounds

The ethyl acetate extract of *G. nepalense* was isolated by repeated column chromatography (Sephadex LH-20 and silica gel) to afford six pure compounds. These compounds were subjected to <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, and ESI-MS analyses for structure identification (Data are shown in the Appendix). They are elucidated to be kaempferol (**1**) (7-9), kaempferol-7-*O*-β-D-glucopyranoside (**2**) (10-12), quercetin-7-*O*-α-rhamnopyranoside (**3**) (13), pyrogallol (**4**) (14), gallic acid (**5**) (15), and epipinoresinol (**6**) (16) according to their <sup>1</sup>H- and <sup>13</sup>C-NMR spectral data and compared with spectral values in literatures. This is the first report for the isolation of compounds **1-6** (Figure 1) from this plant.

**Table 1. Anti-inflammatory activity of GN fractions of *G. nepalense***

Treatment	Rats (individuals)	Dose (g/kg)	Weight of ear edema (mg)
Model	15	0.0	7.33 ± 3.04
Aspirin	15	0.6	2.84 ± 1.94**
GN-EA	15	2.5	2.48 ± 2.02**
GN-Bu	15	2.5	3.36 ± 1.76**
GN-W	15	7.5	5.62 ± 2.86 <sup>#</sup>

All values are expressed as mean of 15 mice in each group. Statistically significant: \*\*  $p < 0.01$  compared to control, <sup>#</sup> compared to GN-EA.



**Figure 1. The chemical structures of compounds 1-6.**

Previously studies have suggested that the ethyl acetate extract of *G. carolinianum* was the anti-inflammatory active fraction (17). The polyphenolic compounds of *Geranium* spp. such as flavonoids and tannins have been shown to possess free radical scavenging/antioxidant anti-inflammatory activity both *in vivo* and *in vitro* (12,18,19,20). The anti-inflammatory effect of *G. nepalense* was evaluated by TPA-induced ear edema model *in vivo* in this study. The results showed that both GN-EA and GN-Bu fractions inhibited TPA-induced inflammation. Six compounds were isolated from the GN-EA fraction. The chemical structures of them were elucidated to be flavonoids (1-3), pyrogallol (4), gallic acid (5), and epipinoresinol (6), which all belong to polyphenolic. 1 isolated from *Hibiscus cannabinus* L. showed significant anti-inflammatory (21). 3 and 6 were also isolated from the leaves of *Brasenia schreberi*, and both compounds exhibited anti-inflammatory activities (22). Our results suggested that highly-enriched 4 and 5 may be the best active constituents related to the traditional utilization of this herb.

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#### Appendix

##### <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, and MS analyses of the isolated compounds 1-6.

**Kaempferol (1)**, yellow powder, C<sub>15</sub>H<sub>10</sub>O<sub>6</sub>, ESI-MS: *m/z* 285 [M - H]<sup>-</sup>. <sup>1</sup>H-NMR (400 Hz, DMSO-*d*<sub>6</sub>) δ: 7.98 (d, *J* = 8.8, H-2', 6', 2H), 6.96 (d, *J* = 8.8, H-3', 5', 2H), 6.74 (d, *J* = 2.0, H-8), 6.36 (d, *J* = 2.0, H-6). <sup>13</sup>C-NMR (100 Hz, DMSO-*d*<sub>6</sub>) δ: 150.3 (C-2), 138.7 (C-3), 178.4 (C-4), 161.4 (C-5), 92.3 (C-6), 160.2 (C-7), 97.7 (C-8), 160.9

(C-9), 105.2 (C-10), 130.5 (C-1'), 130.2 (C-2', 6'), 115.6 (C-3', 5'), 155.3 (C-4').

*Kaempferol-7-O-β-D-glucopyranoside (2)*, C<sub>21</sub>H<sub>20</sub>O<sub>11</sub>, ESI-MS: *m/z* 447 [M – H]<sup>–</sup>. <sup>1</sup>H-NMR (400 MHz, CD<sub>3</sub>OD) δ: 8.25 (d, *J* = 8.8, H-2', 6', 2H), 7.73 (d, *J* = 8.8, H-3', 5', 2H), 6.25 (d, *J* = 2.1, H-8), 6.19 (d, *J* = 2.1, H-6), 5.82 (d, *J* = 9.7, glc-H-1). <sup>13</sup>C-NMR (100 MHz, CD<sub>3</sub>OD) δ: 146.8 (C-2), 138.7 (C-3), 176.4 (C-4), 161.4 (C-5), 98.7 (C-6), 164.2 (C-7), 93.7 (C-8), 157.1 (C-9), 104.6 (C-10), 132.0 (C-1'), 128.6 (C-2', 6'), 115.4 (C-3', 5'), 155.6 (C-4'), 103.8 (glc-C-1), 75.0 (glc-C-2), 78.6 (glc-C-3), 71.3 (glc-C-4), 79.0 (glc-C-5), 62.4 (glc-C-6).

*Quercetin-7-O-α-rhamnopyranoside (3)*, C<sub>21</sub>H<sub>20</sub>O<sub>11</sub>, ESI-MS: *m/z* 447 [M – H]<sup>–</sup>; <sup>1</sup>H-NMR (400 MHz, CD<sub>3</sub>OD) δ: 7.33 (d, *J* = 1.5 Hz, H-2'), 6.90 (d, *J* = 8.3 Hz, H-5'), 7.29 (dd, *J* = 1.5, 8.3 Hz, H-6'), 6.19 (br. s, H-6), 6.35 (br. s, H-8), 5.34 (br. s, rha-H-1), 0.93 (d, *J* = 6.1 Hz, rha-H-6); <sup>13</sup>C-NMR (100 MHz, CD<sub>3</sub>OD) δ: 148.9 (C-2), 136.2 (C-3), 179.6 (C-4), 158.5 (C-5), 99.8 (C-6), 165.8 (C-7), 94.7 (C-8), 158.2 (C-9), 105.9 (C-10), 133.0 (C-1'), 116.4 (C-2'), 146.4 (C-3'), 147.8 (C-4'), 117.0 (C-5'), 123.9 (C-

6'), 103.5 (rha-C-1), 71.9 (rha-C-2), 72.0 (rha-C-3), 72.1 (rha-C-4), 73.3 (rha-C-5), 17.6 (rha-C-6).

*Pyrogallol (4)*, brown needle, C<sub>6</sub>H<sub>6</sub>O<sub>3</sub>, EI-MS *m/z* (%): 126 (100, M<sup>+</sup>); <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ: 6.79 (t, *J* = 8.0, H-5), 6.63 (d, *J* = 8.0, H-4, 6, 2H); <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ: 110.7 (C-4, C-6), 120.7 (C-5), 140.0 (C-2), 147.8 (C-1, 3).

*Gallic acid (5)*, colorless needles, C<sub>7</sub>H<sub>6</sub>O<sub>5</sub>, EI-MS *m/z* (%): 170 (100, M<sup>+</sup>), 153 (80), 126 (92); <sup>1</sup>H-NMR (400 MHz, C<sub>5</sub>D<sub>5</sub>N) δ: 8.08 (s, H-2, H-6); <sup>13</sup>C-NMR (100 MHz, C<sub>5</sub>D<sub>5</sub>N) δ: 123.0 (C-1), 110.7 (C-2, C-6), 147.8 (C-3, C-5), 140.7 (C-4), 169.9 (C-7).

*Epipinoresinol (6)*, white powder, C<sub>20</sub>H<sub>22</sub>O<sub>6</sub>, ESI-MS: *m/z* 357 [M – H]<sup>–</sup>; <sup>1</sup>H-NMR (400 MHz, C<sub>5</sub>D<sub>5</sub>N) δ: 4.94 (d, *J* = 3.3, H-7, 7', 2H), 3.23 (m, H-8, 8', 2H), 4.31 (m, H-9a, 9'a, 2H), 4.00 (m, H-9b, 9'b, 2H), 3.76 (s, C-3, 3'-OMe, 6H); <sup>13</sup>C-NMR (100 MHz, C<sub>5</sub>D<sub>5</sub>N) δ: 133.2 (C-1, 1'), 111.0 (C-2, 2'), 148.9 (C-3, 3'), 147.9 (C-4, 4'), 116.5 (C-5, 5'), 119.8 (C-6, 6'), 86.5 (C-7, 7'), 54.8 (C-8, 8'), 72.0 (C-9, 9'), 58.2 (C-3, 3'-OMe).