Preparative separation and quantitative determination of two kaurenoic acid isomers in root barks of *Acanthopanax gracilistylus*

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**[ABSTRACT]** The kaurenoic acid-type diterpenoids in *Acanthopanax Cortex* have been reported to be the major active components. However, the diterpenoids are present as position isomers that exacerbate the challenges in obtaining standards compounds. Little work has been done on the quantitative analysis of the diterpenoids in the herb. In the present study, two diterpenoid isomers ent-16βH,17-isovalerate-kauran-19-oic acid (1) and ent-16βH,17-methyl butanoate-kauran-19-oic acid (2) with high purity were separated by analytical HPLC, followed by recrystallization in acetone. Furthermore, an HPLC-ELSD method was developed and validated for simultaneous determination of 1 and 2 in 9 batches of *Acanthopanax Cortex* samples. The HPLC separation and quantification was achieved in 40 min using an Agela Promosil C18 column eluted with a gradient of water and acetonitrile. The calibration curves showed good linearity ($R^2 \geq 0.9999$) within the test ranges. The LOD ranged from 0.407 to 0.518 μg and LOQ ranged from 1.018 to 1.295 μg. The precisions (%RSD) were within 1.47% for the two isomers. The recovery of the assay was in the range of 98.78%–99.11% with RSD values less than 2.76%. It is the first time to establish a quantitative HPLC method for the analysis of the bioactive kaurenoic acid isomers in the herb.

**[KEY WORDS]** *Acanthopanax gracilistylus*, Kaurenoic Acid; Quantification; HPLC-ELSD

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**Introduction**

*Acanthopanax gracilistylus* W. W. Smith is a woody medicinal plant found in Asia such as China, Japan and South Korea. Its root bark (*Acanthopanax Cortex*), named *Wujiapi* in Chinese, is a well-known traditional Chinese medicine (TCM) and has been used for the treatment of paralysis, arthritis, rheumatism, lameness and high blood pressure [1]. It is also used as *Wujiapi* healthcare liquor to strengthen bones and muscles [2]. However, this herb is easily confused with *Periploca Cortex* (the root barks of *Periploca septum* Bunge) by appearance and similar clinical actions. The adulterant and misuse of *Acanthopanax Cortex* may cause severe toxic and cardiotoxic effects due to the presence of large amounts of cardiac glycosides in *Periploca Cortex* [3]. Phytochemical studies on *Acanthopanax Cortex* are very limited and only few lignans (e.g., sesamin, ariensin, savinin, and acanthosides B and D) [4-5], diterpenoids (e.g., pimarane and kaurane) [6-7], triterpenoids (e.g., chisanoside, eluterosides I, K, L, and M) [8-9], sterols (e.g., β-sitosterol, stigmasterol and campesterol) [8] and flavonoids (e.g., hyperin) [10], have been reported. Among these phytochemicals, the diterpenoid ent-kaurenoic acid derivatives have been found to possess anti-inflammatory [11], anti-tumor [12-13], anti-HIV [14], anti-obese, vascular smooth muscle contraction inhibitory [15] and immunosuppressive activities [16]. Therefore, this type of components could be potentially used as important chemical markers for quality evaluation of this herb [17]. However, the quality control of *Acanthopanax Cortex* has not been established because the majority of these compounds standards are commercially unavailable. In addition, the diterpenoids in this herb such as ent-16βH,17-isovalerate-kauran-19-oic acid and ent-16βH,17-methyl butanoate-kauran-19-oic acid occur as isomers, which exacerbates the challenge of preparative purification and identification [18]. Therefore, in the present study, two ent-kaurenoic acid isomers ent-16βH,17-isovalerate-kauran-19-oic acid (1) and ent-16βH,17-methyl butanoate-kauran-19-oic acid (2) were prepared and purified by preparative HPLC and validated by analytical HPLC, followed by recrystallization in acetone. Furthermore, an HPLC-ELSD method was developed and validated for simultaneous determination of the two isomers. The precisions (%RSD) were within 1.47% for the two isomers. The recovery of the assay was in the range of 98.78%–99.11% with RSD values less than 2.76%. It is the first time to establish a quantitative HPLC method for the analysis of the bioactive kaurenoic acid isomers in the herb.

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ran-19-oic acid (2) were isolated preparatively from Acanthopanacis Cortex by RP-HPLC for the first time. Their structures were identified by NMR and MS data, and verified by X-ray crystallographic analysis. In addition, the cytotoxic activity of 1 and 2 against four human cancer cell lines were evaluated. A HPLC-ELSD method for simultaneous quantitative determination of these two isomers in Acanthopanacis Cortex was also established and proposed as its quality control method for the first time.

**Materials and Methods**

**General procedures**

$^3$H- and $^{13}$C NMR spectra were recorded on a Bruker DRX400 spectrometer (Bruker BioSpin Corporation, Billerica, MA, USA) using TMS as an internal standard. ESI-MS spectra were measured on a Velos Pro dual-pressure linear ion trap mass spectrometer (Thermo Scientific, San Jose, CA, USA). Silica gel (100–200 mesh) was purchased from Qingdao Marine Chemical Co., Ltd. (Qingdao, China). Acetonitrile and methanol were of HPLC grade and obtained from J&K Scientific Ltd. (Beijing, China). Deionized water was obtained from a Milli-Q system (Milli pore, Bedford, MA, USA). Ethanol, petroleum ether and acetone were of analytical grade, and were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

**Plant materials**

Nine batches of Acanthopanacis Cortex were collected from Tianjin, Hebei, Hubei, Anhui and Guangdong Provinces of China. All the samples were authenticated by Dr. CHEN Dao-Feng, and the voucher specimen (WJP-1101) has been deposited at the Department of Pharmacognosy, School of Pharmacy, Fudan University, Shanghai, China.

**Instrumentation and chromatographic conditions**

The preparative separation conditions were as follows: the separation was carried out on an Agilent 1200 series HPLC system equipped with a photodiode array (PDA) detector, a quaternary pump and column thermostat (Agilent, Santa Clara, CA, USA); the diterpenoids were separated on a Promosil C$_{18}$ column (250 mm × 4.6 mm, 5 μm) (Bonna-Agela Technologies, Tianjin, China) at a temperature of 30 °C, with water–acetonitrile (70 : 30) as the mobile phase; and the wavelength for the detection was set at 210 nm.

Quantitative determination conditions were as follows: HPLC analysis was also carried out on the above Agilent 1200 series HPLC coupled to an evaporative light scattering detector (ELSD, Agilent, Santa Clara, CA, USA) under similar experimental conditions except for the mobile phase and the detector; the mobile phase was acetonitrile (A) and water (B) with a gradient program as follows: 0–20 min, isocratic 77% A; 20–60 min, linear gradient 77%–70% A at a flow rate of 1.0 mL min$^{-1}$; ELSD used nitrogen as nebulizing gas with a pressure of 3.33 bar, and the temperature of the drift tube was set at 45 °C.

**Extraction, isolation, and preparative purification of two ent-kaurenoic acid isomers from Acanthopanacis Cortex**

The dried root barks of _Acanthopanax gracilistylus_ (12 kg) were powdered and extracted three times with 95% aqueous ethanol (30 L) at 70 °C for 4 h. The ethanolic extracts were combined and evaporated to dryness under reduced pressure to yield a dark brown residue (1.2 kg). Part of the ethanolic extract (1.0 kg) was suspended in 2.4 L of water and partitioned 5 times with equal volumes of petroleum ether (PE, 60–90 °C). The PE extract was concentrated under reduced pressure to afford the PE fraction (300 g). Part of the PE fraction (150 g) was subjected to column chromatography over silica gel eluting with PE, then PE–acetone (50 : 1), and finally PE–acetone (30 : 1). The PE–acetone (30 : 1) fraction was collected and prepared in methanol for preparative separation by the above HPLC system with a C$_{18}$ column. The peaks with retention times at 30 and 32 min were individually collected, concentrated, and recrystallized from acetone at 4 °C, affording Compounds 1 (200 mg) and 2 (300 mg). The purities of these two compounds were determined to be over 98% by HPLC-ELSD analysis.

**X-ray data of Compounds 1 and 2**

Suitable crystals for the X-ray diffraction were obtained by recrystallization from the acetone solutions of 1 and 2. The crystallographic data of 1 and 2 were collected on a Bruker APEX2 CCD diffractometer (Bruker AXS Inc., Madison, WI, USA). Crystal data for 1: C$_{25}$H$_{40}$O$_{4}$, $M_r$ = 404.57, crystal size 0.25 × 0.20 × 0.13 mm$^3$, orthorhombic, space group P2$_1$2$_1$2$_1$, $a$ = 10.072 3(8) Å, $b$ = 11.084 1(7) Å, $c$ = 42.136 3(8) Å, $α$ = 90°, $β$ = 90°, $γ$ = 90°, $V$ = 4 704.2(5) Å$^3$, $T$ = 296(2) K, $Z$ = 8, $D_{calc}$ = 1.143 mg/m$^3$, $F(000)$ = 1 776, 35 913 reflections collected ($θ_{max}$ = 66.96°), 8140 independent reflections ($R_{int}$ = 0.025 8). Crystal data for 2: C$_{25}$H$_{40}$O$_{4}$, $M_r$ = 404.57, crystal size 0.26 × 0.18 × 0.13 mm$^3$, orthorhombic, space group P2$_2$1$_2$1$_2$, $a$ = 10.084(2) Å, $b$ = 11.238(2) Å, $c$ = 41.452(8) Å, $α$ = 90°, $β$ = 90°, $γ$ = 90°, $V$ = 4 697.6(16) Å$^3$, $T$ = 296(2) K, $Z$ = 8, $D_{calc}$ = 1.144 mg/m$^3$, $F(000)$ = 1 776, 22 186 reflections collected ($θ_{max}$ = 66.99°), 8143 independent reflections ($R_{int}$ = 0.0231). Their crystallographic data have been deposited in the Cambridge Crystallographic Data Centre (CCDC) with the accession CCDC 1493354 and 1493353 for 1 and 2, respectively. These data can be obtained free of charge from CCDC via 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44-1223-336-033 or e-mail: deposit@ccdc.cam.ac.uk).

**Cytotoxic activity of two ent-kaurenoic acid isomers**

The human cancer cell lines, including lung cancer cell line (A549), prostate cancer cells (DU145), epidermoid carcinoma of the nasopharynx (KB), and oral epidermoid carcinoma resistant cells (Kbvin), were obtained from American Type Culture Collection (ATCC, Rockville, MD, USA). The cytotoxicity was measured according to our previously published method [19]. The concentration of test compounds resulting in 50% growth inhibition (IC$_{50}$) was estimated. Paclitaxel was used as the positive control. All experiments were
performed in three independent replicates and the IC\textsubscript{50} values are expressed as the mean ± SD.

**Standard and sample solution preparation for quantitative analysis**

A standard mixed stock solution of 1 and 2 was made in HPLC-grade methanol at a concentration of 0.577 and 1.038 mg·mL\(^{-1}\), respectively. A series of working standard solutions of these two isomers with different concentrations were prepared by stepwise dilution of the mixed stock solution.

The dried Acanthopanacis Cortex samples were ground to pass through a 60-mesh sieve. Each sample (0.4 g) was accurately weighed and extracted with 40 mL of cyclohexane by ultrasonication at room temperature for 50 min. The extract was filtered, and 20 mL of the successive filtrate was then evaporated to dryness under reduced pressure. The residue was re-dissolved in methanol, transferred into a 2-mL calibrated flask, and made up to exactly 2 mL with methanol. After filtration through a 0.45-\(\mu\)m filter, the successive filtrate was analyzed by the HPLC system. All the samples were analyzed in triplicate.

**Results and Discussion**

**Preparative separation of the isomeric diterpenoids from Acanthopanacis Cortex**

Due to the presence of another predominant diterpenoid (ent-kaur-16-en-19-oic acid) as the impurity\(^{[17]}\), it must be carefully removed from the PE fraction by eluting this fraction through a silica gel column with PE−acetone (50 : 1). The two isomers were enriched in the eluates from the PE−acetone (30 : 1) in 4.9% overall yield starting from the crude drug materials. TLC analysis of this fraction containing the isomers showed that only a purple dense spot can be observed by spraying with 10% H\(_2\)SO\(_4\) in ethanol. Attempts to separate the isomers by repeated silica gel CC with different solvent systems or Sephadex LH-20 were unsuccessful. Fortunately, the two isomers could be well separated by a routine analytical C\textsubscript{18} HPLC column eluted with acetonitrile–water (30 : 70), followed by a recrystallization from acetone to obtain high purities of compounds 1 (200 mg, ca. 0.196% yield) and 2 (300 mg, ca. 0.294% yield). It should be noted that, although the two isomers could not achieve baseline separation (R > 1.5) under the isocratic elution condition, this purification step facilitated the subsequent recrystallization process through enrichment of the individual isomers.

**Identification of the isomeric diterpenoids**

The identification of two compounds was carried out by NMR and MS analysis. Compounds 1 and 2 were identified as ent-16βH,17-isovalerate-kauran-19-oic acid and ent-16βH,17-methyl butanoate-kauran-19-oic acid (Fig. 1), respectively, by comparison with the literature data\(^{[18]}\). Their structures and relative configurations were further confirmed by X-ray analysis for the first time (Fig. 2). These two compounds were ent-kaurane-type diterpenoid isomers with different methyl-substituted positions on the side chain (Fig. 1).

**Fig. 1** Structures of 1 (ent-16βH,17-isovalerate-kauran-19-oic acid) and 2 (ent-16βH,17-methyl butanoate-kauran-19-oic acid)

**Fig. 2** The ORTEP views of the crystal structures of 1 (ent-16βH,17-isovalerate-kauran-19-oic acid) and 2 (ent-16βH,17-methyl butanoate-kauran-19-oic acid)
29.0, 184.4, 15.8, 173.7, 43.8, 26.0, 22.7, 22.6 (C<sub>1-20</sub>, 1<sup>β</sup>, 2); ESI-MS m/z 403 [M − H]<sup>−</sup>. These data were in good agreement with the reported compound ent-16/βH,17-isovalerate-kauran-19-oic acid<sup>[18]</sup>.

<sup>1</sup>-ent-16/βH,17-methyl butanoate-kauran-19-oic acid (2): colorless flaky crystals (acetone); mp: 169–171 °C; [α]<sub>D</sub><sup>20</sup> −63.3° (c 1.0, MeOH); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ: 3.87 (2H, d, J = 7.4 Hz, H-17), 2.37 (1H, m, H-2′), 1.24 (3H, s, H-18), 1.13 (3H, d, J = 7.0 Hz, H-5′); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ: 40.9, 19.3, 38.0, 43.9, 57.2, 22.6, 41.8, 45.1, 55.5, 39.8, 19.0, 31.4, 38.8, 37.4, 45.2, 39.8, 68.5, 29.2, 184.5, 15.8, 177.3, 41.4, 27.0, 11.9, 16.9 (C<sub>1-20</sub>, 1<sup>β</sup>); ESI-MS m/z 403 [M − H]<sup>−</sup>. These data were in good agreement with the reported compound ent-16/βH,17-methyl butanoate-kauran-19-oic acid<sup>[18]</sup>.

Cytotoxic activity of two ent-kauronic acid isomers

For the first time, the cytotoxic activity of the two isomers were tested against A549, Du145, KB, and Khvin cancer cell lines using the sulforhodamine B (SRB) assay and paclitaxel as the positive control. The results expressed as IC<sub>50</sub> values are summarized in Table 1. Both the isomers showed moderate cytotoxic activities against all the selected cancer cell lines, with IC<sub>50</sub> values being 14.47–32.73 μg mL<sup>−1</sup> for 1, and 17.02–26.36 μg mL<sup>−1</sup> for 2, respectively.

**Table 1** Cytotoxicity of compounds 1 and 2 against four cancer cell lines (IC<sub>50</sub>, μg·mL<sup>−1</sup>)

<table>
<thead>
<tr>
<th>Compound</th>
<th>A549</th>
<th>DU145</th>
<th>KB</th>
<th>Khvin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14.47 ± 0.23</td>
<td>15.78 ± 0.09</td>
<td>18.45 ± 0.14</td>
<td>32.73 ± 0.20</td>
</tr>
<tr>
<td>2</td>
<td>17.02 ± 0.18</td>
<td>18.84 ± 0.11</td>
<td>26.36 ± 0.12</td>
<td>24.55 ± 0.16</td>
</tr>
<tr>
<td>Paclitaxel&lt;sup&gt;þ&lt;/sup&gt;</td>
<td>0.006</td>
<td>0.005</td>
<td>0.007</td>
<td>3.56</td>
</tr>
</tbody>
</table>

<sup>þ</sup> Positive control. 1 and 2 represented ent-16/βH,17-isovalerate-kauran-19-oic acid and ent-16/βH,17-methyl butanoate-kauran-19-oic acid, respectively.

Optimization of extraction procedure

In the present study, the effects of extraction solvents and extraction methods on the extraction efficiency for target compounds were investigated. Both methanol and cyclohexane were tested for their efficiency as extraction solvents, and cyclohexane was found a better solvent with higher recovery and less impurity (data not shown). Extraction methods including ultrasonication (15, 30, 50, and 60 min) and heat refluxing (30, 50, and 60 min) were studied for extraction efficiency using cyclohexane as extraction solvent. The extracted efficiency could reach the threshold in both extraction methods with extraction times over 50 min. Ultrasonication could afford slightly higher extraction efficiency with the same extraction time (data not shown). In the present study, ultrasonication for 50 min was selected as the extraction method due to its advantages of technical simplicity, convenience and efficiency. In addition, the solid/liquid ratio (mass/volume, g·mL<sup>−1</sup>, 1 : 50–1 : 200) on the extraction efficiency was also tested under ultrasonication. The results showed that a solid/liquid ratio of 1 : 100 was the best for extracting both isomers based on the HPLC results (data not shown).

Optimization of chromatographic conditions

In the present study, several HPLC conditions including mobile phase compositions and types of stationary phase were studied for optimization of separation of these two isomers. Several types of HPLC columns such as Agela Promosil C<sub>18</sub> column (4.6 mm × 250 mm, 5 μm), Phenomenex Kinetex C<sub>18</sub> (100 mm × 4.6 mm, 2.6 μm), Thermo Scientific Hypercarb C<sub>18</sub> (150 mm × 4.6 mm, 5 μm), and Ultimate Pentafluorophenyl (PFP) column (250 mm × 4.6 mm, 5 μm) were compared in term of the peak resolution. Finally, the Agela Promosil C<sub>18</sub> column was selected due to its higher resolution (R > 1.5) for the two isomers. The effects of mobile phase compositions and elution gradients on the chromatographic separation were then studied. The separation with ACN–H<sub>2</sub>O system is superior to methanol–H<sub>2</sub>O system. Addition of a chiral resolving agent (β-cyclodextrin) into the mobile phase couldn’t improve the peak resolution. As a result, a good separation of these two isomers was achieved with a gradient elution of 0.1% aqueous formic acid and acetonitrile. As shown in Fig. 3, a baseline separation was achieved within 40 min under the optimized conditions, with symmetrical, sharp and well-resolved peaks for the two isomers. A typical HPLC-ELSD chromatogram is shown in Fig. 3. It is worth to note that two peaks at around 26.0 min retention time in Fig. 3 were tentatively assigned to acanthoic acid<sup>[20]</sup> and ent-kaur-16-en-19-oic acid<sup>[17]</sup>, the latter of which was confirmed by comparison of the HPLC retention time and UV profile with the authentic standard.

Fig. 3 Typical HPLC-ELSD chromatograms of the cyclohexane extract of an Acanthopanacis Cortex sample from No. 7 sample (A), and the two isomer standards, 1 (B), and 2 (C). 1 and 2 represented ent-16/βH,17-isovalerate-kauran-19-oic acid and ent-16/βH,17-methyl butanoate-kauran-19-oic acid, respectively.
**Method validation**

The calibration curve of each compound was established by analyzing the potential linear relationship between logarithm values of peak area (lgY) and analytes masses (lgX, μg). Table 2 lists the linear calibration curves with their correlation coefficients $r^2$, linear range, limit of detection (LOD) and limit of quantification (LOQ) of each standard determined. The result suggested good correlations ($r^2 \geq 0.9999$) between the standard masses and their peak areas in test ranges. The LOD and LOQ values for each analyte were determined experimentally from the injection of the diluted standard solution and calculated using the minimum concentration of analyte providing signal-to-noise ratios (S/N) of 3 and 10, respectively. The LOD values ranged from 0.407 2 to 0.518 0 μg, and the LOQ values ranged from 1.018 0 to 1.295 0 μg.

The intra-day precisions of the analytic method were determined by analyzing six replicates of every individual standard sample. Analytes 1 and 2 represent *ent-16β/H,17-isovalerate-kauran-19-oic acid* and *ent-16β/H,17-methyl butanoate-kauran-19-oic acid*, respectively. The lgY and lgX in linear equations represent the logarithm values of the peak area and the analyte masses (μg), respectively.

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Linear equation</th>
<th>$r^2$</th>
<th>Linear range (μg)</th>
<th>LOD (μg)</th>
<th>LOQ (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>lgY = 1.484 8 lgX + 2.049 1</td>
<td>0.999 9</td>
<td>1.15–11.54</td>
<td>0.407 2</td>
<td>1.018 0</td>
</tr>
<tr>
<td>2</td>
<td>lgY = 1.460 1 lgX + 2.061 3</td>
<td>0.999 9</td>
<td>2.08–20.76</td>
<td>0.518 0</td>
<td>1.295 0</td>
</tr>
</tbody>
</table>

Analytes 1 and 2 represent *ent-16β/H,17-isovalerate-kauran-19-oic acid* and *ent-16β/H,17-methyl butanoate-kauran-19-oic acid*, respectively. The lgY and lgX in linear equations represent the logarithm values of the peak area and the analyte masses (μg), respectively.

**Quantitative analysis of the isomers in Acanthopanacis Cortex**

In the present study, nine samples of Acanthopanacis Cortex were analyzed in triplicate, using the newly developed HPLC-ELSD method and the results are summarized in Table 4. The contents ranged from 0.146% to 0.304% for 1 and from 0.147% to 0.462% for 2, respectively.

<table>
<thead>
<tr>
<th>No.</th>
<th>Collecting place</th>
<th>Origin place</th>
<th>1 (% of total)</th>
<th>2 (% of total)</th>
<th>Total (% of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Wuhan, Hubei</td>
<td></td>
<td>0.245 ± 0.002 2</td>
<td>0.360 ± 0.007 5</td>
<td>0.605 5 ± 0.006 2</td>
</tr>
<tr>
<td>2</td>
<td>Yingcheng, Hubei</td>
<td></td>
<td>0.170 ± 0.003 4</td>
<td>0.203 ± 0.001 2</td>
<td>0.373 6 ± 0.004 0</td>
</tr>
<tr>
<td>3</td>
<td>Bozhou, Anhui</td>
<td>Guangxi</td>
<td>0.2651 ± 0.005 5</td>
<td>0.315 0 ± 0.009 9</td>
<td>0.580 0 ± 0.015 0</td>
</tr>
<tr>
<td>4</td>
<td>Guangzhou, Guangdong</td>
<td>Anhui</td>
<td>0.146 1 ± 0.001 9</td>
<td>0.147 0 ± 0.002 8</td>
<td>0.293 1 ± 0.004 2</td>
</tr>
<tr>
<td>5</td>
<td>Bozhou, Anhui</td>
<td>Hubei</td>
<td>0.244 1 ± 0.004 9</td>
<td>0.462 3 ± 0.005 9</td>
<td>0.706 4 ± 0.010 6</td>
</tr>
<tr>
<td>6</td>
<td>Tianjin</td>
<td></td>
<td>0.304 1 ± 0.005 0</td>
<td>0.374 9 ± 0.002 7</td>
<td>0.679 0 ± 0.007 7</td>
</tr>
<tr>
<td>7</td>
<td>Anguo, Hebei</td>
<td>Xiaogan, Hubei</td>
<td>0.229 8 ± 0.004 8</td>
<td>0.414 8 ± 0.005 1</td>
<td>0.644 6 ± 0.009 9</td>
</tr>
<tr>
<td>8</td>
<td>Anguo, Hebei</td>
<td>Wuhu, Anhui</td>
<td>0.293 2 ± 0.004 1</td>
<td>0.388 6 ± 0.010 5</td>
<td>0.681 8 ± 0.014 6</td>
</tr>
<tr>
<td>9</td>
<td>Anguo, Hebei</td>
<td>Waxian, Sichuan</td>
<td>0.200 4 ± 0.004 5</td>
<td>0.300 3 ± 0.003 9</td>
<td>0.500 7 ± 0.008 3</td>
</tr>
</tbody>
</table>
individual contents of 1 and 2 differed in different batches of samples. The content of 2 was higher than that of 1 in all samples, with the highest content found in No. 5 sample and the lowest in No. 4 sample. The total contents of the isomers ranged from 0.293 1% to 0.706 4%. The total content in No. 5 sample was 2.41 times higher that of No. 4 sample (Table 4), indicating that the content of two compounds varied greatly.

These data, together with the cytotoxic activity of the two compounds, suggested that quality control for the commercial products of the herb are urgently required.

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References


