In Vitro Antitumor Activities of Biologically Active Extracts and Isolated Phytochemicals From *Rabdosia latifolia* C. Y. Wu Et H. W. Li in Addenda

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Abstract
To investigate the antitumor activities, as well as phytochemical constituents of *Rabdosia latifolia* C. Y. Wu et H. W. Li in Addenda (*R. latifolia*) extracts, the light petroleum, dichloromethane, ethyl acetate, *n*-butanol, and water extracts from *R. latifolia* were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays on MCF-7, HepG2, A549, Hela, and BGC-823 cells. We further examined cell cycle and apoptosis of the biologically active extracts on MCF-7 cells. The constituents of the biologically active extracts were determined using ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry (UPLC-Triple-TOF/MS) and gas chromatography–mass spectrometric (GC–MS) analysis. MTT assays showed that the dichloromethane extract had an inhibitory effect on MCF-7 and BGC-823 cells and that at 50 µg/mL showed 9.9% ± 1.7% inhibition of MCF-7 cells and 81.7% ± 9.5% of BGC-823 cells, respectively. The data for cell cycle analysis clearly showed a significant block in the S-phase. The dichloromethane extract induced apoptosis of MCF-7 cells, and the cell apoptosis rate showed time and concentration effects. UPLC-Triple-TOF/MS analysis revealed the presence of 16 structurally characterized compounds in the dichloromethane extract, all of which were identified for the first time in this species. Ent-kauranoids, such as henryin (9) and its analogs, were the predominant compounds. Subsequently, GC–MS analysis showed the presence of 20 volatile compounds, including γ-sitostenone, β-amyrone, γ-tocopherol, and dotriacontane. Based on the results of this study and previous literature reports, it is concluded that the ent-kauranoids, such as henryin, were the predominant components of *R. latifolia* with antitumor activity.

Keywords
*Rabdosia latifolia*, anti-tumor, extracts, ent-kauranoid

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Introduction
*Rabdosia latifolia* C. Y. Wu et H. W. Li in Addenda (*R. latifolia*), a perennial herb in the Labiatae family, is primarily distributed throughout the east district of Sichuan Province and the north district of Guizhou Province in China. The herb has been commonly applied in folk medicine for nasal obstruction, headache, colds, and has significant effects. Previous studies have revealed that *R. latifolia* is rich in essential trace elements, including iron, zinc, magnesium, boron, and copper. Simultaneously, the chemical constituents of light petroleum extracts (PEE) of *R. latifolia* were analyzed by gas chromatography–mass spectrometry (GC–MS). The results showed 48 components from the non-methylated samples, including 22 esters, 18 terpenes and their derivatives, 6 hydrocarbons, and 2 steroids. Among them, the relative content of terpenoids and their derivatives accounted for 54.8% of the total outflow peak area, and the contents of α-amyrone (18.1%), γ-sitostenone (15.9%), and β-amyrone (15.3%) were abundant. Meanwhile, 17 ingredients were identified from the methylated samples, and the relative content of methyl oleate...
and methyl palmitate exceeded 15%. During the past 4 decades, phytochemical studies on the Labiatae family have demonstrated that ent-kauranoids are the characteristic and main compounds exhibiting a broad spectrum of bioactivities, such as antitumor and antibacterial properties. To investigate further the value of R. latifolia, the antitumor activities of light PEE, dichloromethane extract (DCME), ethyl acetate extract (EAA), n-butanol extract (NBE), and water extract (WE) of R. latifolia on MCF-7, HepG2, A549, Hela, and BGC-823 cells were tested. The phytochemical constituents of the biologically active extracts were characterized by ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-Triple-TOF/MS) and GC–MS.

**Materials and Methods**

**Materials and Instruments**

*Raphosia latifolia* C. Y. Wu et H. W. Li in Addenda, family Labiatae, was obtained from Daozhen County, Guizhou Province, China, and identified by Dr Li Jixin (Guizhou University of Traditional Chinese Medicine). The specimen (code: ZMPC-LGP-001) was stored at Zunyi Medical and Pharmaceutical College.

Human breast adenocarcinoma cell line MCF-7, human hepatocellular carcinoma cell line HepG2, human non-small lung carcinoma cell line A549, human epithelial carcinoma cell line HeLa, and human gastric carcinoma cell line BGC-823 were obtained from the China Center for Type Culture Collection in Wuhan University. Dulbecco’s Modified Eagle Medium, fetal bovine serum, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cells were cultured in 90% RPMI-1640 medium, fetal bovine serum, and 5% CO2 at 37°C.

**Preparation of Different Extracts**

Concisely, 50 g of *R. latifolia* was placed in a round-bottomed flask, and 250 mL of light petroleum was added and heated for reflux extraction, for 2 hours. The liquid was then cooled and filtered. Subsequently, 200 mL of light petroleum was added to the residue and refluxed for 1.5 hours, cooled, and filtered again. The light PEE (5.35 g) was obtained by mixing and concentration of the 2 extraction solutions. Afterward, a similar process was used to obtain DCME (3.21 g), EAE (4.96 g), NBE (1.2 g), and WE (4.28 g) in turn.

**MTT Assay**

Five tumor cell lines (MCF-7, HepG2, A549, Hela, and BGC-823) were plated in the appropriate media on 96-well plates in a 100 µL total volume at an optimum density. Quintuplicate wells were treated with media and 5 extracts (PEE, DCME, EAE, NBE, WE; DMSO as diluent) at different concentrations (50, 20, 2 µg/mL). The plates were incubated at 37°C in 5% CO2 for 24 hours. Cell viability was determined based on the mitochondrial conversion of MTT to formazan. The absorbance (A) was measured at 562 nm. Inhibition rate (%) was calculated using the following equation:

\[
\text{Inhibition rate } (\%) = \frac{A_{\text{Control}} - A_{\text{Treated}}}{A_{\text{Control}}} \times 100\%
\]

**Cell Cycle Analysis of the Biologically Active Extracts**

According to a previous report, MCF-7 cells (1 × 10⁶ cells/mL) were seeded in T25 culture flasks and grown for 6 hours to reach 80%-90% confluency. Cells were treated with DCME at 0, 2, 20, and 50 µg/mL. DMSO (final concentration <0.1%) was used as a negative control. Camptothecin (20 µM, concentration equivalent to 7.0 µg/mL) was used as a positive control to ensure the reliability of the experiment and prevent the presence of extracts without effect to determine whether the experiment is normal. It is not to compare camptothecin with the DCME extracts of *R. latifolia*. After treatment for either 24, 48, or 72 hours at 37°C, floating and adherent cells were collected, washed twice with phosphate-buffered saline (PBS), and fixed in 70% ethanol at 4°C overnight. The cells were then collected and resuspended in PBS with RNase (25 µg/mL) and propidium iodide (PI, 50 µg/mL) for 30 minutes at 37°C. The stained cells were analyzed using Epics XL flow cytometry (Beckman Coulter, USA).

**Apoptosis Assay of the Biologically Active Extracts**

Briefly, MCF-7 cells (1 × 10⁶ cells/mL) were seeded and grown to reach 80%-90% confluency. Cells were treated with DCME at 0, 2, 20, and 50 µg/mL for 24, 48, or 72 hours. Camptothecin (20 µM) as a positive control and DMSO as a negative control. The cells were collected and evaluated using an Annexin V-fluorescein isothiocyanate (FITC)/PI Apoptosis Kit (Beckman Coulter, USA), according to the manufacturer’s protocols. The cells were analyzed using Epics XL flow cytometry (Beckman Coulter, USA).
percentage of apoptotic cells was determined using Mcycle software.

**Phytochemical Analysis of the Biologically Active Extracts by LC–MS/MS**

LC conditions: sample was separated on an Acquity UPLC HSS T3 (150 mm × 3.0 mm i.d., 1.7 µm, Waters, USA) analytical column maintained at 50°C. 0.1% Formic acid–water (H₂O) was used for phase A, and 0.1% formic acid–acetoniitrite for phase B, to enhance linear gradient elution at, 0 minutes 5% B; 15 minutes 40% B; 33 minutes 95% B; 36 minutes 95% B; the flow rate was 0.3 mL/min; detection wavelength was 254 nm, and injection volume 5 µL.

MS chromatographic conditions: UPLC-Triple-TOF 5600+ TOF-MS; in positive and negative ion scanning modes; scanning range: m/z 100-2000; at optimized gas (GS₃): 55 psi; atomized gas (CUR): 35 psi; ion source temperature (TEM): 550°C (negative), 600°C (positive); ion source voltage (IS): −4500 V (negative); first-order scanning: declustering voltage (DP): 100 V; focus voltage (CE): 10 V; second-order scanning: MS data were acquired under TOF-MS-Product Ion-IDA mode, with the CID energies of -20, −40, and −60 V. The mass axis was corrected using a CDS pump before sampling, to lower mass axis error to less than 2 ppm.

**Phytochemical Analysis of the Biologically Active Extracts by GC–MS**

GC–MS analysis of the biologically active extracts from *R. latifolia* was performed using an Agilent 5975C mass spectrometer coupled to an Agilent HP6890 gas chromatograph. An aliquot of the extract was dissolved in dichloromethane and injected into the GC–MS apparatus. Next, the data were displayed on a FB-5MS column, 30 m in length, 0.25 mm i.d., and 0.25 µm in thickness. The carrier gas was helium. The initial temperature of the GC oven was 46°C (retaining 2 minutes), warming to 118°C at 8°C/minute, and then to 310°C at 6°C/minute (maintaining for 11 minutes). The injector and detector temperatures were set at 280°C and 250°C, respectively. The mass range was scanned from 29 to 500 amu. The identification of compounds was based on a comparison of their mass spectra with those of the NIST2014 and Wiley 275 mass spectral libraries.

**Results and Discussion**

**MTT Assay**

The inhibitory activities on MCF-7, HepG2, A549, Hela, and BGC-823 cells of PEE, DCME, EAE, NBE, and WE from *R. latifolia* are shown in Table 1. This shows that DCME had the best activity against the 5 cell lines, especially MCF-7 and BGC-823 cells. At 50 µg/mL concentration, the inhibition rate of DCME on MCF-7 and BGC-823 cells reached 79.9% ± 1.7%

| Table 1. Inhibitory Activities of Different Extracts From *Rabdosia latifolia* on 5 Cell Lines (x ± s, N = 3) |
|---|---|---|---|---|---|
| Extracts | Concentration (µg/mL) | MCF-7 cells | HepG2 cells | Hela cells | BGC-823 cells | A549 cells |
| PEE | 50 | 0a | 4.7 ± 4.7 | 1.7 ± 4.8 | 0 | 0 |
| | 20 | 0 | 7.4 ± 1.0 | 5.8 ± 5.5 | 0 | 0 |
| | 2 | 0 | 5.7 ± 1.2 | 7.5 ± 1.4 | 0 | 0 |
| DCME | 50 | 79.9 ± 1.7 | 52.5 ± 6.0 | 46.7 ± 5.1 | 81.7 ± 9.5 | 62.3 ± 2.0 |
| | 20 | 55.8 ± 2.6 | 23.2 ± 3.7 | 23.1 ± 0.9 | 71.2 ± 7.7 | 36.7 ± 7.7 |
| | 2 | 27.2 ± 4.8 | 9.7 ± 0.6 | 21.4 ± 1.1 | 33.5 ± 3.2 | 8.6 ± 2.8 |
| EAE | 50 | 0 | 12.6 ± 5.8 | 6.1 ± 1.5 | 36.0 ± 5.8 | 0 |
| | 20 | 0 | 12.9 ± 0.7 | 10.7 ± 0.9 | 3.4 ± 2.9 | 1.0 ± 6.0 |
| | 2 | 0 | 2.9 ± 2.8 | 11.7 ± 5.3 | 0 | 2.2 ± 1.3 |
| NBE | 50 | 0 | 0 | 6.7 ± 2.0 | 0 | 0 |
| | 20 | 0 | 3.2 ± 0.6 | 0 | 0 | 0 |
| | 2 | 0 | 2.2 ± 3.6 | 0 | 0 | 0 |
| WE | 50 | 0 | 0 | 0 | 0 | 0 |
| | 20 | 0 | 0 | 0 | 0 | 0 |
| | 2 | 0 | 2.1 ± 2.1 | 0 | 0 | 0 |
| Doxorubicin | 5.8 (1 × 10⁻⁷ mol/L) | 79.3 ± 6.2 | 66.2 ± 2.3 | 42.1 ± 1.8 | 63.5 ± 3.4 | 56.0 ± 9.9 |
| | 2.9 (5 × 10⁻⁷ mol/L) | 62.3 ± 5.3 | 43.3 ± 6.4 | 35.1 ± 5.6 | 41.2 ± 5.3 | 37.0 ± 14.9 |
| | 0.58 (1 × 10⁻⁸ mol/L) | 56.2 ± 8.9 | 17.2 ± 2.2 | 22.0 ± 3.5 | 19.2 ± 5.6 | 22.1 ± 2.8 |

DCME, dichloromethane extract; EAE, ethylacetate extract; PEE, petroleum extract; NBE, n-butanol extract; WE, water extract.
aNo inhibition. Doxorubicin as a positive control.
and 81.7% ± 9.5%, respectively. The inhibitory effect of EAE on HepG2, A549, Hela, and BGC-823 cells was very weak and there was no inhibition of MCF-7 cells. The PEE, NBE, and WE of *R. latifolia* had no inhibitory effect on the 5 cell lines. In other words, the antitumor activity of *R. latifolia* was mainly concentrated in the DCME.

**Cell Cycle Analysis of the Biologically Active Extracts**

To determine whether the decreased cell number caused by treatment with DCME was related to cell cycle arrest, we assessed the effect of DCME on cell cycle perturbation by flow cytometry. The data in Table 2 clearly show a significant block in S-phase of the cell cycle. After treatment of MCF-7 cells for 24, 48, or 72 hours with DCME, the proportion of S-phase cells increased and the number of G2 phase cells decreased compared with the control group. Compared with 7 µg/mL of camptothecin, 50 µg/mL of DCME had a stronger inhibition of S-phase cells.

### Table 2. Cell Distribution in Different Phase of MCF-7 Cells Induced by DCME From *Rabdosia latifolia*

| Group       | Concentration (µg/mL) | 24 hours | 48 hours | 72 hours |
|-------------|-----------------------|----------|----------|----------|
|             | G1 (%) | S (%) | G2 (%) | G1 (%) | S (%) | G2 (%) | G1 (%) | S (%) | G2 (%) |
| DCME        | 50     | 64.6  | 20.6   | 13.8    | 62.3  | 22.9  | 12.9  | 63.3  | 31.4  | 4.3   |
|             | 20     | 61.5  | 17.5   | 19.4    | 61.2  | 21.0  | 16.9  | 61.0  | 21.6  | 15.5  |
|             | 2      | 63.7  | 19.7   | 16.2    | 62.7  | 19.2  | 17.1  | 63.7  | 20.0  | 15.0  |
| Camptothecin| 7      | 62.0  | 19.9   | 17.5    | 62.0  | 19.9  | 17.5  | 66.1  | 19.8  | 13.8  |
| Control     | 0a     | 66.1  | 17.1   | 15.8    | 64.8  | 17.1  | 17.0  | 63.7  | 17.4  | 18.0  |

DCME, dichloromethane extract.

*0.1% Dimethyl sulfoxide.

**Apoptosis Assay of Biologically Active Extracts**

In order to determine whether DCME could induce cell apoptosis, Annexin V-FITC/PI was used in DCME-treated MCF-7 cells and the control. MCF-7 cells treated with increasing concentrations of DCME exhibited a significant increase in the apoptotic cell fraction, indicating apoptosis induction (Figures 1 and 2). Compared with the control group, DCME induced apoptosis of MCF-7 cells, and the cell apoptosis rate showed a time and concentration effect. DCME (50 µg/mL) was more capable of causing apoptosis of MCF-7 cells after 24, 48, and 72 hours than camptothecin.

**LC–MS/MS Analysis of the Biologically Active Extracts**

According to the results of the antitumor activity screening, DCME was the most biologically active extract. UPLC-Triple-TOF/MS technology was used to analyze the composition of DCME from *R. latifolia*. Current ion chromatograms were

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**Figure 1.** Effect of ethylacetate extract on apoptosis of MCF-7 cells. MCF-7 cells were untreated and treated with increasing concentrations of dichloromethane extract (DCME) for 24, 48, and 72 hours.
acquired in positive and negative ion modes. The law of mass spectral fragmentation in the current ion signals was used, and data were retrieved using Scifinder and Reaxy databases. Sixteen compounds were identified: 12 ent-kauranoids, 2 flavonoids, and 2 phenylalanine derivatives (Table 3 and Figure 3). The ent-kauranoids were macrocalyxin G \(1^1\), pharicin W \(2^6\), taibaijaponicains C \(3^{10}\), adenolin E \(4^{11}\), sodoponin \(5^{12}\), effusanin B \(6^{11}\), moecrystal E \(7^{14}\), henryin \(9^{16}\), rosthornin A \(11^{18}\), and melissoidesin P \(12^{29}\). The 2 flavonoids were cirsiliol \(13^{20}\) and cirsilineol \(14^{21,22}\), and the 2 phenylalanine derivatives were aurantiamide \(15^{23}\) and patriscabratine \(16^{24}\). These compounds were identified from \textit{R. latifolia} for the first time. According to the UPLC results, the henryin \(9\) content reached 64.3%. Combining the findings of this article with previous literature reports, \(25–27\) henryin and other ent-kauranoids were found to be the main constituents in the DCME of \textit{R. latifolia} displaying inhibitory activities on MCF-7 and BGC-823 cells. The contents of rosthornin A \(11\) and melissoidesin P \(12\) reached 2.90% and 1.55%, respectively. The contents of cirsiliol \(13\), cirsilineol \(14\), aurantiamide \(15\), and patriscabratine \(16\) were each less than 1%.

### Table 3. Liquid Chromatography–Mass Spectrometry (MS)/MS Data and Contents of Dichloromethane Extract of \textit{R. latifolia}

| No. | \(t_R\) (min) | Fragment ions \((m/z)\) | Identification | Relative content (%) |
|-----|---------------|--------------------------|----------------|---------------------|
| 1\(^a\) | 9.91 | 426.2209 [M], 425.2166 [M – H], 407.2059 [M – 18 –H], 365.1974 [M – 60 – H] | Macrocalyxin G | 0.34 | 28 |
| 2\(^a\) | 10.93 | 454.2157 [M], 453.2119 [M – H], 391.2136 [M – 62 – H], 331.1915 313.1802, 59.0172 | Pharicin W | 0.16 | 6 |
| 3\(^a\) | 11.92 | 410.2252 [M], 409.2220 [M – H], 391.2120 [M – 18 – H], 331.1907, 313.1797, 59.0173 | Taibaijaponicains C | 0.07 | 10 |
| 4\(^a\) | 11.23 | 437.2165 [M – H], 419.2079 [M – 18 – H], 333.2066, 315.1950, 59.0179 | Adenolin E | 0.08 | 11 |
| 5\(^a\) | 12.15 | 407.2055 [M – H], 389.2108 [M – 18 – H], 299.1643, 281.1554, 59.0176 | Sodoponin | 0.23 | 12 |
| 6\(^a\) | 12.42 | 391.2118 [M + H], 373.2111 [M + H – 18], 313.1792 [M + H – 18 – 60] | Effusanin B | 0.41 | 13 |
| 7\(^a\) | 12.92 | 393.2262 [M + H], 375.2162 [M + H – 18], 315.1946 [M + H – 18 – 60], 297.1841 | Moecrystal E | 1.22 | 14 |
| 8\(^a\) | 13.00 | 405.1905 [M – H], 387.1804 [M – H – 18], 319.1897, 301.1792, 59.0172 | Lasiokaurin | 0.51 | 15 |
| 9\(^a\) | 12.63 | 375.2167 [M + H – 18], 315.1954 [M + H – 18 – 60] | Henryin | 64.31 | 16 |
| 10\(^a\) | 15.11 | 413.1930 [M + Na], 373.2008 [M + H – 18], 285.1475 [M + H – 18 – 28 – 60] | Pseurata C | 0.67 | 17 |
| 11\(^a\) | 14.63 | 359.2205 [M + H-18], 299.1999 [M + H – 18 – 60] | Rosthornin A | 2.90 | 18 |
| 12\(^a\) | 12.97 | 377.2315 [M + H – 18], 317.2096 [M + H – 18 – 60] | Melissoidesin P | 1.55 | 19 |
| 13\(^a\) | 15.36 | 331.0810 [M + H], 316.0565 [M + H – 15], 298.0465 [M + H – 15 – 18] | Cirsiliol | 0.90 | 20 |
| 14\(^a\) | 18.11 | 345.0965 [M + H], 330.0725 [M + H – 15], 312.0620 [M + H – 15 – 18] | Cirsilineol | 0.59 | 21,22 |
| 15\(^a\) | 18.76 | 403.1996 [M + H], 385.1900, 224.1060, 152.1066, 91.0566, 77.0412 | Aurantiamide | 0.21 | 23 |
| 16\(^a\) | 21.72 | 445.2121 [M + H], 224.1062 [M + H – 120], 91.0550, 77.0406 | Patriscabratine | 0.63 | 24 |

\(t_R\), retention time.

\(^a\)Compound detected for the first time.
**GC–MS Analysis of the Biologically Active Extracts**

GC–MS analysis of the DCME from *R. latifolia* enabled the identification of 20 compounds (Table 4) belonging to different chemical families. The DCME contained 46.4% of dotriacontane, which was the major volatile compound in this extract. The terpenoids represented 16.5%, consisting mainly of:

- **24.16** Neophytadiene
- **24.31** Hexahydrofarnesyl acetone
- **32.25** 4,8,12,16-Tetramethylheptadecan-4-olide
- **36.52** Heptacosane
- **38.46** α-Tocospiro A
- **38.78** α-Tocospiro B
- **40.98** γ-Tocopherol
- **43.16** Dotriacontane
- **44.71** β-Amyrone
- **45.18** α-Amyrone
- **45.75** γ-Sitostenone

**Table 4. Chemical Composition of Dichloromethane Extract of Rabdosia latifolia Analyzed by Gas Chromatography–Mass Spectrometry.**

| No. | t<sub>r</sub> (min) | Identification                  | Mol. formula | RI values in NIST 2014 | R. content (%) |
|-----|-------------------|--------------------------------|--------------|------------------------|----------------|
| 17  | 5.91              | 2,6-Dimethylpyridine           | C<sub>7</sub>H<sub>9</sub>N | 878                    | 0.022          |
| 18  | 7.99              | 2-Amylfuran                    | C<sub>9</sub>H<sub>14</sub>O | 993                    | 0.073          |
| 19  | 10.25             | Nonanal                        | C<sub>9</sub>H<sub>18</sub>O | 1104                   | 0.14           |
| 20  | 11.13             | 4-Ketoisophorone               | C<sub>9</sub>H<sub>12</sub>O<sub>2</sub> | 1144                  | 0.031          |
| 21  | 12.16             | Melilotal                      | C<sub>9</sub>H<sub>10</sub>O  | 1183                   | 0.095          |
| 22  | 12.23             | Decanal                        | C<sub>9</sub>H<sub>16</sub>O<sub>2</sub> | 1206                  | 0.23           |
| 23  | 13.39             | Ethylmethylmaleimide           | C<sub>6</sub>H<sub>9</sub>NO<sub>2</sub> | 1239                  | 0.27           |
| 24  | 18.55             | 4-Acetylguaiacol               | C<sub>9</sub>H<sub>10</sub>O<sub>3</sub> | 1489                  | 0.32           |
| 25  | 19.01             | Dihydroactinidolide            | C<sub>11</sub>H<sub>16</sub>O<sub>2</sub> | 1532                  | 0.38           |
| 26  | 24.16             | Neophytadiene                  | C<sub>20</sub>H<sub>38</sub>  | 1837                   | 1.51           |
| 27  | 24.31             | Hexahydrofarnesyl acetone      | C<sub>18</sub>H<sub>36</sub>O | 1844                   | 4.12           |
| 28  | 32.25             | 4,8,12,16-Tetramethylheptadecan-4-olide | C<sub>21</sub>H<sub>40</sub>O<sub>2</sub> | 2364                  | 0.27           |
| 29  | 36.52             | Heptacosane                    | C<sub>21</sub>H<sub>46</sub>  | 2700                   | 0.26           |
| 30  | 38.46             | α-Tocospiro A                  | C<sub>29</sub>H<sub>50</sub>O<sub>4</sub> | 2860                  | 0.43           |
| 31  | 38.78             | α-Tocospiro B                  | C<sub>29</sub>H<sub>50</sub>O<sub>4</sub> | 2882                  | 0.71           |
| 32  | 40.98             | γ-Tocopherol                   | C<sub>29</sub>H<sub>48</sub>O<sub>2</sub> | 3065                  | 0.24           |
| 33  | 43.16             | Dotriacontane                  | C<sub>32</sub>H<sub>66</sub>  | 3200                   | 46.41          |
| 34  | 44.71             | β-Amyrone                      | C<sub>30</sub>H<sub>52</sub>O  | 3337                   | 7.08           |
| 35  | 45.18             | α-Amyrone                      | C<sub>30</sub>H<sub>52</sub>O  | 3376                   | 1.72           |
| 36  | 45.75             | γ-Sitostenone                  | C<sub>29</sub>H<sub>48</sub>O  | 3483                   | 5.24           |

RI, retention index.
of β-amyrone (7.1%) and hexahydrofarnesyl acetone (4.1%). The aldehyde and aceto phenone derivatives represented 0.79%, along with other minor constituents. According to our knowledge, the chemical composition of DCME from *R. latifolia*, investigated by GC–MS, was performed for the first time in this study.

**Conclusions**

So far, *R. latifolia* has not been studied. We first found by MTT analysis that the antitumor extract was the dichloromethane fraction. Then, 16 compounds were separated and identified by UPLC-Triple-TOF/MS; 19 volatile components were detected by GC–MS from the dichloromethane extract. Based on these results and those reported in the literature, we suggest that the ent-kaurenoi ds, such as henryin, might be the predominant antitumor constituents of *R. latifolia*. Our findings also enrich our knowledge of the phytochemical content of *R. latifolia*.

**Declaration of Conflicting Interests**

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**Supplemental Material**

Supplemental material for this article is available online.

**References**

1. Zhang LB, Zhu GH, ZY W, et al. Flora of China. Science Press; 1977:524.
2. Liang GP, JX L, Qiu J. Determination of trace elements in *Rabdosa latifolia* by ICP-MS. *Mod Agr Sci & Tecb*. 2019;4:222-225.
3. Wu J, Yang J, Zhang J. GC-MS Analysis of petroleum ether extracts from *Rabdosa latifolia*. *Fine Chem Intermed*. 2019;49(3):39-42.
4. Zhu L, Huang S-H, Yu J, Hong R, et al. Constructive innovation in three compositae plants by LC/DAD-APCI/MS. *Chin Med*. 2016;11(1):1-16. doi:10.1186/s13020-016-0120-y
5. Jin Y, Tian T, Ma Y, et al. Chemical profiling and total quality assessment of *Isodon japonica* using data-independent acquisition mode combined with superimposed multiple product ion UHPLC-Q-TOF-MS and chemometric analysis. *RSC Adv*. 2019;9(3):1403-1418. doi:10.1039/C8RA08732F
6. YZ L, Chen YZ. Diterpenoids from *Rabdosa pseudo-irrorata*. *J Nat Prod*. 1990;53(4):841-844.
7. Zeng S, Liu W, Nie F-F, et al. LGY-202, a new flavonoid with a piperazone substitution, shows antitumor effects in vitro and in vivo. *Biochem Biophys Res Commun*. 2009;385(4):551-556. doi:10.1016/j.bbrc.2009.05.099
8. Chen H-S, Bai M-H, Zhang T, Li G-D, Liu M. Ellagic acid induces cell cycle arrest and apoptosis through TGF-β/Smad3 signaling pathway in human breast cancer MCF-7 cells. *Int J Onmol*. 2015;46(4):1730-1738. doi:10.3892/ijo.2015.2870
9. Du J, Sun Y, Lu Y-Y, et al. Berberine and evodiamine act synergistically against human breast cancer MCF-7 cells by inducing cell cycle arrest and apoptosis. *Anticancer Res*. 2017;37(11):6141-6151. doi:10.21873/anticancerres.12063
10. Li BL, Talbajaponicains C. Talbajaponicains C and D: two new diterpenoids from *Isodon japonica*. *Planta Med*. 2002;68(5):477-479. doi:10.1055/s-2002-32080
11. Zhang RP, Zhang HJ, Lin ZW, et al. STRUCTURES OF EFFUSANINS, ANTIBACTERIAL DITERPENOIDS FROM *RABDOSIA EFFUSA*. *Chem Lett*. 1980;9(12):1635-1638. doi:10.1246/cl.1980.1635
12. CB L, Sun HD, Zhou J, maocrystals Sof. new diterpenoids from *Rabdosa ericophylla*. *Acta Chim Sinica*. 1988;46(7):657-662.
13. Wong LL, Liang Z, Chen H, Zhao Z, et al. Rapid differentiation of Xihuangeao from the three *Isodon* species by UPLC-ESI-QTOF-MS/MS and chemometrics analysis. *Clin Med*. 2016;11(1):1-16. doi:10.1186/s13020-016-0120-y
14. Jin Y, Tian T, Ma Y, et al. Chemical profiling and total quality assessment of *Isodon japonica* using data-independent acquisition mode combined with superimposed multiple product ion UHPLC-Q-TOF-MS and chemometric analysis. *RSC Adv*. 2019;9(3):1403-1418. doi:10.1039/C8RA08732F
15. YL X, Chen YZ. Diterpenoids from *Rabdosa pseudo-irrorata*. *J Nat Prod*. 2019;53(4):841-844.
16. YL X, YB M. Diterpenoid constituents from *Rabdosa rathbornii*. *Phytochemistry*. 1989;28(11):3235-3237.
17. Chao A-H, Han Q-B, Li R-T, et al. Diterpenoids from *Isodon meliosides*. *J Nat Prod*. 2004;67(9):1441-1444. doi:10.1021/np030418l
18. Lai J-P, Lin YH, Su J, Shen H-M, Ong CN. Identification and characterization of major flavonoids and caffeoylquinic acids in *Centaurea napifolia* plant. *Bull Chem Pharm Bull*. 2002;50(5):1039-1041. doi:10.1248/bpb.29.1039
19. Akkal S, Benayache F, Bentamene A, et al. Flavonoid aglycones from *Centauraea napifolia*. *Chem Nat Compd*. 2003;39(2):219-220. doi:10.1023/A:1024834518756
23. Tang J, Tewtrakul S, Wang Z, et al. Aurantiamide acetate from stems of *Zanthoxylum dissitum* Hemsley. *J Chin Pharm sci*. 2003;12(4):231-233.

24. Yuan L, Wang JH, Sun TM. Total synthesis and anticancer activity studies of the stereoisomers of asperphenamate and patriscabratine. *Chin Chem Lett*. 2010;21(2):155-158. doi:10.1016/j.ccl.2009.10.004

25. Li X, Pu J, Jiang S, et al. Henryin, an ent-kaurane diterpenoid, inhibits Wnt signaling through interference with β-catenin/Tcf4 interaction in colorectal cancer cells. *PLoS One*. 2013;8(7):e68525 doi:10.1371/journal.pone.0068525

26. Dai L-P, Li C, Yang H-Z, et al. Three new cytotoxic ent-kaurane diterpenes from *Isodon excisoides*. *Molecules*. 2015;20(9):17544-17556. doi:10.3390/molecules200917544

27. Li X-N, Pu J-X, Du X, et al. Structure and cytotoxicity of diterpenoids from *Isodon eriocalyx*. *J Nat Prod*. 2010;73(11):1803-1809. doi:10.1021/np1004328

28. Wang XR, Wang HP, HP H, et al. Structures of macrocalyxin B, F, G and H, and maoyerabdosin from *Isodon macrocalyx*. *Phytochemistry*. 1995;38(4):921-926. doi:10.1016/0031-9422(94)00474-8