SUPPLEMENTARY MATERIAL

Chemical composition, antioxidant, antimicrobial and anticancer activities of the essential oil from the rhizomes of *Zingiber striolatum* Diels

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ABSTRACT

The chemical composition and biological activities of the essential oil (EO) from the rhizomes of *Zingiber striolatum* Diels were reported for the first time. Forty-five compounds were identified, and represented 95.7% of the total composition of the EO. The predominant components of the EO were \(\beta\)-phellandrene (24.0%), sabinene (17.3%), \(\beta\)-pinene (11.4%), geranyl linalool (8.6%), terpinen-4-ol (8.3%), \(\alpha\)-pinene (5.6%) and crypton (4.5%). The EO revealed a weak DPPH and ABTS radical-scavenging activity. The EO exhibited significant antimicrobial activity with the inhibition zones (12.86–24.62 mm) and MIC (0.78–3.12 mg/mL) against *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans*. The EO showed significant cytotoxicity against human leukemic (K562), lung cancer (A549) and prostatic carcinoma (PC-3) cell lines with the IC\(_{50}\) values of 29.06, 48.37 and 86.05 \(\mu\)g/mL, respectively. Thus, the EO could be regarded as a bioactive natural product with potential for utilization in the cosmetic and pharmaceutical industry.

Key words: *Zingiber striolatum* Diels; essential oil; GC-MS; antioxidant activity; antibacterial activity; anticancer activity.

EXPERIMENTAL

Plant material

*Z. striolatum* was collected from Guizhou Province of China in September 2016. Identity of the species was confirmed by Prof. Shenghua Wei of Guizhou University of Chinese Medicine. A voucher specimen (NO.1936) was deposited at Guizhou Engineering Center for Innovative Traditional Chinese Medicine and Ethnic Medicine, Guizhou University.

Isolation of essential oil

The underground parts of the dry *Z. striolatum* (500 g) were subjected to hydrodistillation for 5 h using a Clevenger-type apparatus to obtain the essential oil (2.25 g, 0.45% w/w). The EO was dried over anhydrous Na\(_2\)SO\(_4\) and stored in an amber bottle at 4°C until further analysis.

Essential oil analysis and identification

Analysis of the EO sample was carried out by an Agilent 6890 gas chromatograph equipped with a
flame ionization detector (FID). Column: capillary column FB-5MSi (30 m × 0.25 mm × 0.25 μm film thickness). The injection volume was 1 μL and split injection was used (split ratio 1:20). The carrier gas Helium was set at 1 mL/min. GC oven temperature was kept at 58°C for 2 min then increased to 160°C at 3°C per min, and programmed to 310°C at a rate of 10°C/min and then finally kept at 310°C for 5 min. The injector temperature was set at 250°C.

GC-MS analyses were performed on Hewlett Packard 6890 gas chromatograph fitted with a FB-5MSi fused silica column, equipped with a Hewlett Packard 5975C mass selective detector. GC parameters were the same as above. Mass spectra were operated in electron ionization mode at 70 eV. Mass range was from m/z 29 to 500.

The percentage of the chemical component of the EO was calculated by the peak area normalization method. The constituents of the EO were identified by their retention time, retention indices relative to n-alkanes (C₉–C₃₀), and as well as by comparison of their mass spectra with those listed in NIST 14 and Wiley 275 mass spectral databases.

Antioxidant activity assay
The antioxidant activity of the EO was assessed by DPPH free radical scavenging assay reported by Lim et al. (2007) with minor modification. 3 mL of the methanol solution of DPPH (0.1 mM) were added to 1 mL various concentrations of sample and the mixtures were kept at shade for 30 min. The absorbance of the samples was measured by spectrophotometer at 517 nm. The ABTS radical scavenging capacity was determined according to the technique reported by Jena et al. (2017). The ABTS solution was produced by mixing 7 mM ABTS solution and 2.45 mM potassium persulfate and then the mixture was incubated in the dark at room temperature for 16 h. Before the experiments, the ABTS solution was diluted with methanol to obtain an OD of 0.70 ± 0.02 at 734 nm. 3 mL of ABTS solution was mixed with 0.3 mL of various concentrations of sample. Results were expressed as ascorbic acid equivalent antioxidant capacity (AEAC). Each experiment was repeated for three times. The result of antioxidant activity test is given in Table S2.

Antimicrobial activity
The antimicrobial test were performed with five microbial strains of Enterococcus faecalis (ATCC 29212), Staphylococcus aureus (ATCC 6538P), Pseudomonas aeruginosa (CMCC (B) 10104), Escherichia coli (ATCC 25922), Candida albicans (CMCC (F) 98001).

Antimicrobial activities of the EO were assessed using the disc agar diffusion method recommended by Zhang et al. (2017) with minor modification. The EO solution were prepared at 85% (v/v), using Tween 20 as an emulsifier to improve the EO dispersion. Filter paper discs (6 mm in diameter) were impregnated with 20 μL of the EO solution or gentamicin (positive control) and incubated for 24 h at 37°C. The inhibition zone (including the 6 mm disk) was measured and recorded after the incubation time. All the tests were repeated in triplicate.

The MIC values of the EO were determined by the microplate dilution method reported by Zhang et al. (2017) with slight modification. The EO was initially diluted in Tween 20, and later in Mueller-Hinton Broth. The 100 μL of twofold dilution of tested EO was transferred into each well and the inoculum was added to all wells. The final concentration of the bacterial cells in the wells was approximately 5 × 10⁵ CFU/ml. The inoculated microplates were incubated at 37°C for 24 h. 10 μL of resazurin aqueous solution (0.01%) was added to each well as an indicator of microbial
growth. The microplates were incubated for 2 h in the dark at 37°C. Each test was repeated in triplicate. The result of antimicrobial activity test is given in Table S3.

**Anticancer Activity**

Human lung cancer cell line (A549), human prostatic carcinoma cell line (PC-3) and human leukemic cell line (K562) were grown in a humidified incubator at 37°C with 5% CO₂ atmosphere. RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, and antibiotics (100 U/mL of penicillin and 100 U/mL of streptomycin) was used for the cell cultures (Sylvestre et al. 2006). The cytotoxic activity was evaluated by MTT assay with slight modification. The cells were seeded at a density of 5 × 10³ cells per well in 80 μL of culture medium and incubated for 24 h before treatment. The EO was dissolved in DMSO, and afterwards serially double diluted with culture medium for use. The dilutions of the EO were added to the wells (20 μL). The microplates were incubated for 48 h. After incubation, 100 μL of MTT (5 mg/mL in PBS) were added to each well and incubated for 4 h under the same culture conditions. Formazan crystals were dissolved in 150 μL DMSO. The optical density was measured at 490 nm using a microplate ELISA reader. The cytotoxic activity was expressed as the concentration of the EO inhibiting cell growth by 50% (IC₅₀). Each test was repeated in triplicate. The result of cytotoxic activity test is given in Table S4.

Table S1. Chemical composition of the essential oil from the rhizomes of *Z. striolatum*

<table>
<thead>
<tr>
<th>Compounds</th>
<th>RI²</th>
<th>RI³</th>
<th>RT (min)</th>
<th>% Area</th>
<th>Identification²</th>
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<td>trans-Caryophyllene</td>
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<td>Caryophyllene oxide</td>
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<td>Humulene oxide II</td>
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<td>α-Bisabolol</td>
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<td>1685</td>
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<tr>
<td>(E,E)-7,11,15-Trimethyl-3-methylen-hexadeca-1,6,10,14-tetraene</td>
<td>1923</td>
<td>1922</td>
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<td>Geranyl linalool</td>
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<td>2034</td>
<td>43.5</td>
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<td>Total identified</td>
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<td></td>
<td>95.7</td>
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Table S2. Antioxidant activity of the essential oil from the rhizomes of *Z. striolatum*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DPPH IC₅₀ (µg/mL)ᵃ</th>
<th>AEAC (mg/100 g)ᵇ</th>
<th>ABTS IC₅₀ (µg/mL)ᵃ</th>
<th>AEAC (mg/100 g)ᵇ</th>
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</thead>
<tbody>
<tr>
<td>Essential oil</td>
<td>3421.81 ± 18.32</td>
<td>28.70</td>
<td>960.87 ± 16.23</td>
<td>125.51</td>
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<tr>
<td>Ascorbic acid</td>
<td>0.98 ± 0.18</td>
<td>1.21 ± 0.21</td>
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<tr>
<td>BHTᶜ</td>
<td>12.62 ± 1.31</td>
<td>5.43 ± 0.86</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ᵃIC₅₀: The concentration of compound that affords a 50% reduction in the assay, expressed as the mean ± SD of triplicate experiments.
ᵇAEAC (ascorbic acid equivalent antioxidant capacity) = (IC₅₀-AA/ IC₅₀ EO) × 10⁵.
ᶜAscorbic acid and BHT as positive control.

Table S3. Antimicrobial activity of the essential oil from the rhizomes of *Z. striolatum*

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Diameter of the inhibition zones (mm)ᵃ</th>
<th>MIC (mg/mL)ᵇ</th>
</tr>
</thead>
</table>
Table S4. Anticancer activity of the essential oil from the rhizomes of Z. striolatum

| Treatment            | Cell line (IC\textsubscript{50} µg/mL)
|----------------------|--------------------------
|                      | A549\textsuperscript{b}  | PC-3\textsuperscript{c} | K562\textsuperscript{d} |
| Essential oil        | 48.87 ± 1.91             | 86.05 ± 2.34             | 29.67 ± 1.52             |
| Cisplatin (positive control) | 24.83 ± 0.32             | 22.35 ± 0.56             | 21.34 ± 0.64             |

\textsuperscript{a}IC\textsubscript{50}: The concentration of compound that affords a 50% reduction in cell growth (after 48 h of incubation), expressed as the mean ± SD of triplicate experiments.

\textsuperscript{b}Human lung cancer cell line.

\textsuperscript{c}Human prostatic carcinoma cell line.

\textsuperscript{d}Human leukemic cell line.

References


