Supporting Information

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Chemical Constituents and Cytotoxic Activities of Essential Oils from the Flowers, Leaves and Stems of *Zingiber striolatum* Diels

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S1: Experimental Details

S1.1: Essential Oils Extraction

The fresh flowers, leaves and stems of *Z. striolatum* were collected from Guizhou Province of China in September 2018. Dry flowers, leaves and stems were separately obtained by drying in a ventilated oven at a temperature of 50°C for 72 h. The dry *Z. striolatum* flowers, leaves and stems (800 g) were separately subjected to hydrodistillation for 5 h using a Clevenger-type apparatus to obtain the essential oils. The essential oils were dried over anhydrous Na_2SO_4 and stored in amber bottle at 4°C until further analysis.

S1.2: Gas Chromatography (GC) Analysis of Essential Oils

Analysis of the essential oils was carried out by an Agilent 6890 gas chromatograph equipped with a flame ionization detector (FID) and a FB-5MSi capillary column (30 m × 0.25 mm × 0.25 μm film thickness). The carrier gas helium was set at a flow rate of 1 mL/min. The injection volume was 1 μL and split injection was used (split ratio 1:20). 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.

S1.3: Gas Chromatography-Mass Spectrometry (GC/MS) Analysis of Essential Oils

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 (EI) mode at 70 eV with mass range (*m/z* 29 to 500).

S1.4: Identification of Chemical Constituents

The percentage of the chemical component of the essential oils was calculated by the peak area normalization method. The constituents of the essential oils were identified by their retention time, retention indices relative to n-alkanes (C_9–C_{30}), and as well as by comparison of their mass spectra with those listed in literature, NIST 14 and Wiley 275 databases [1-4].

S1.5: Cytotoxic Activity Test

Human leukemic cell line (K562), human prostatic carcinoma cell line (PC-3) and human lung cancer cell line (A549) were maintained in 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). The cells were grown in a humidified incubator at 37°C with 5% CO_2 atmosphere. The cytotoxic activity was evaluated by MTT assay with slight modification [5]. The cells were seeded at a density of 5×10^3 cells per well in 80 μL of culture medium and incubated for 24 h before treatment. The essential oils were dissolved in DMSO, and afterwards serially double diluted with culture medium for use. The dilutions of the essential oils were added to the wells (20 μL). The

microplates were incubated for 48 h. After incubation, the medium was removed and 10 µL of MTT (5 mg/mL in PBS) was added to each well and incubated for 4 h under the same culture conditions. Then, the MTT was removed and 150 µL DMSO was added to each well to solubilize the formazan crystals. After shaking for 10 min at room temperature, the optical density was measured at 490 nm using a microplate spectrophotometer (Bio-Rad Model 680, Hercules, CA, USA). The cytotoxic activity was expressed as the concentration of the essential oils inhibiting cell growth by 50% (IC_{50}). All experiments were performed in triplicate.

S1.6: Statistical Analysis

The results of the tests were carried out in triplicate and expressed as the means ± SD. SPSS software (version 19.0) was used for statistical analysis. Data were compared by oneway analysis of variance (ANOVA) using Tukey's multiple range tests. Differences were considered to be significant at $p < 0.05$ level.

References

[1] R. P. Adams (2001). Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy, *Allured Publ. Corp. Carol Stream, IL.*

[2] T. Üstüner, S. Kordali and A. U. Bozhüyük (2018). Herbicidal and fungicidal effects of *Cuminum cyminum*, *Mentha longifolia* and *Allium sativum* essential oils on some weeds and fungi, *Rec. Nat. Prod.* **12(6)**, 619-629.

[3] J. Chane-Ming, R. Vera and J. C. Chalchat (2003). Chemical composition of the essential oil from rhizomes, leaves and flowers of *Zingiber zerumbet* Smith from Reunion Island, *J. Essent. Oil Res.* **15**, 202-205.

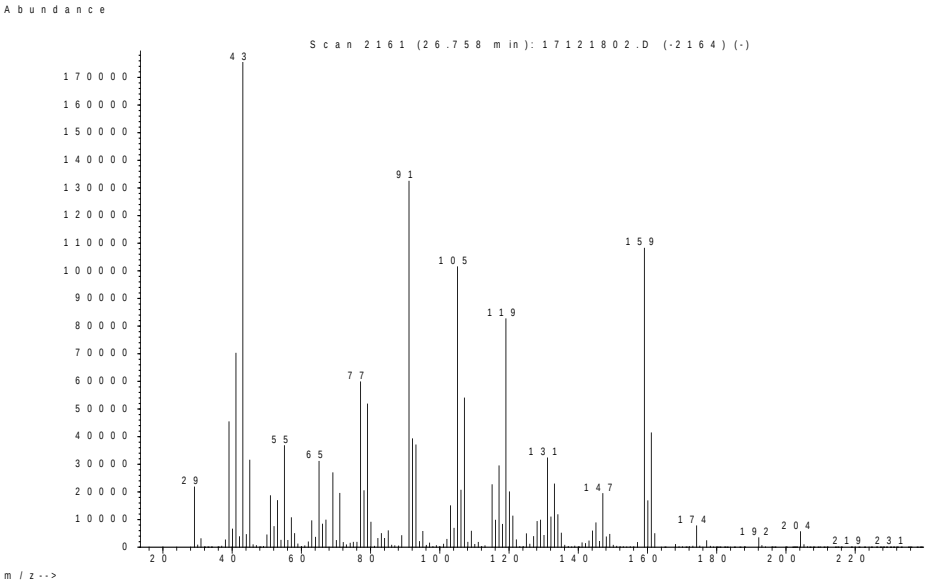
[4] P. Evangelia, V. Constantinos, C. Maria and T. Olga (2017). Study of volatile components of *Acacia farnesiana* Willd. flowers, *Rec. Nat. Prod.* **11(5)**, 474-478.

[5] T. Mosmann (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays, *J. Immunol. Methods* **65**, 55-63.

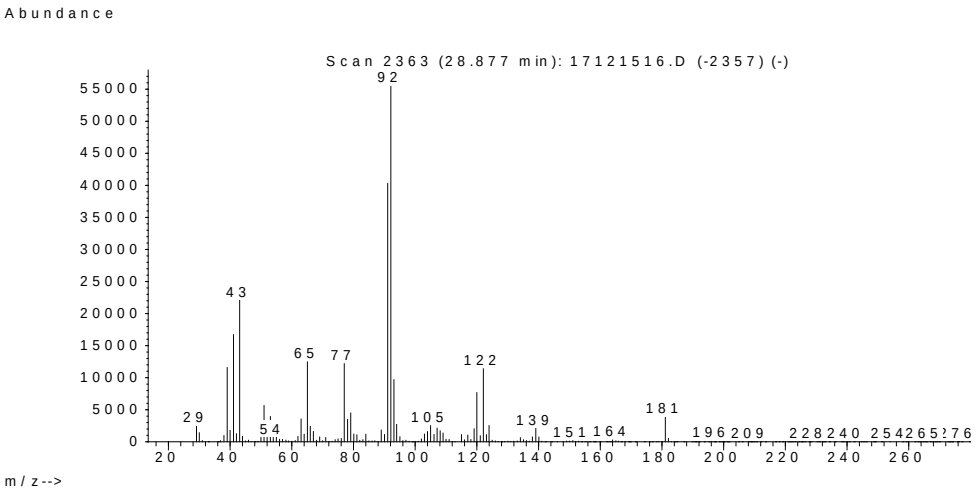
S2: Unknown compounds

S2.1: Table of the unknown compounds

Compounds	RI	% Area		
		flowers	leaves	stems
Unknown compound 1	1392	-	2.4	-
Unknown compound 2	1444	0.8	-	-
Unknown compound 3	1496	-	-	0.7
Unknown compound 4	1514	-	0.4	-
Unknown compound 5	1644	-	1.5	-
Unknown compound 6	1668	0.8	0.4	-
Unknown compound 7	1730	-	0.5	-
Unknown compound 8	1751	1.4	-	-
Unknown compound 9	1884	-	-	1.9
Unknown compound 10	1964	-	-	2.2
Unknown compound 11	2042	-	-	1.5



S2.2: Unknown compound 1



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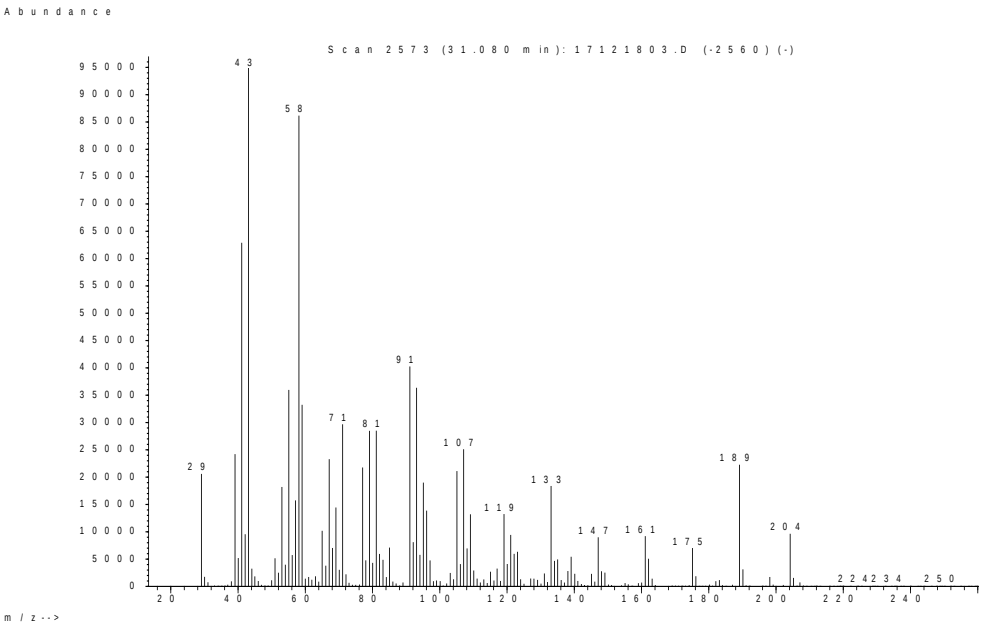

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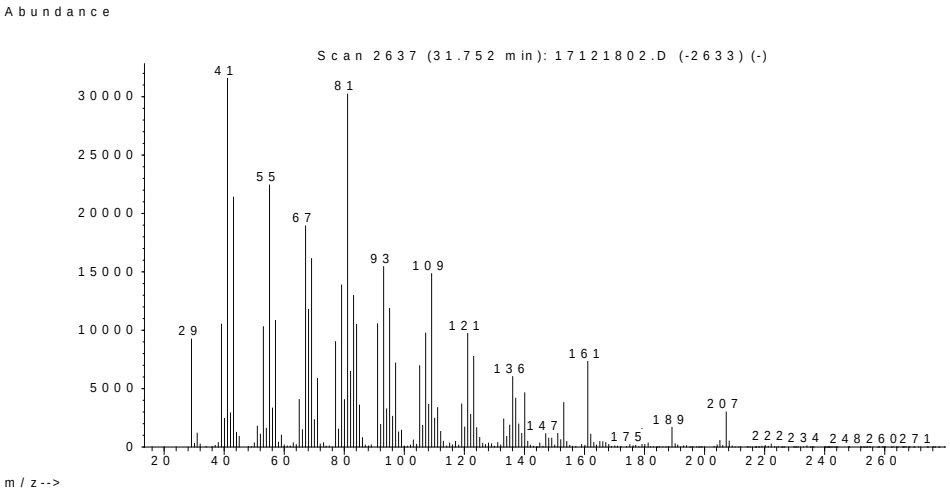

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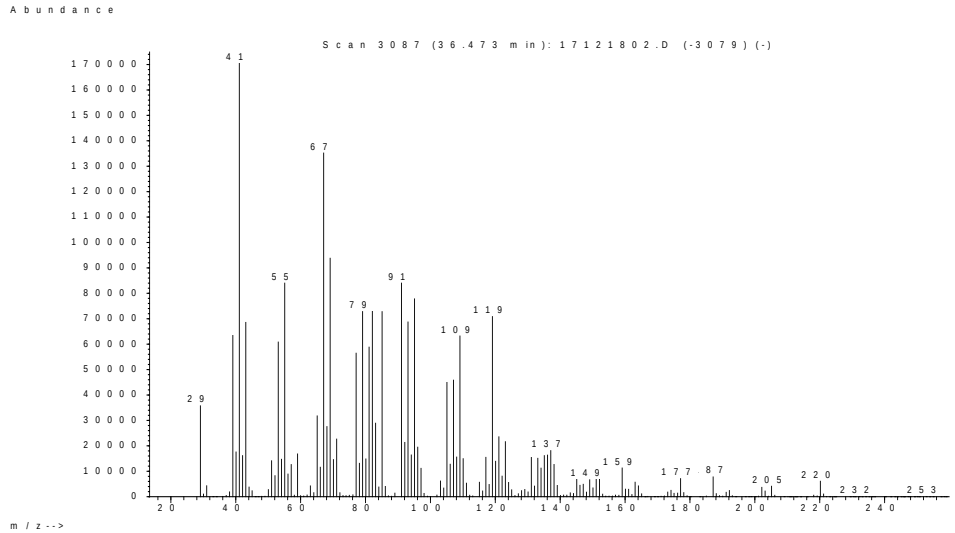

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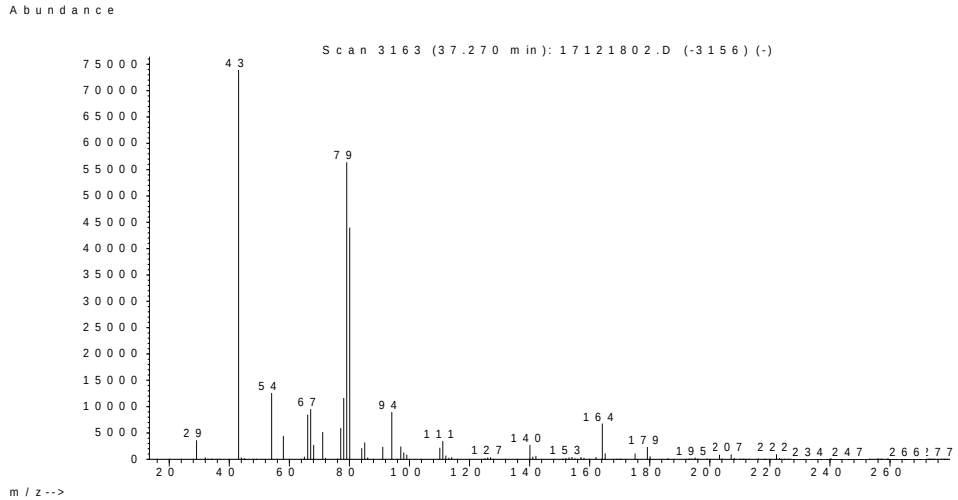

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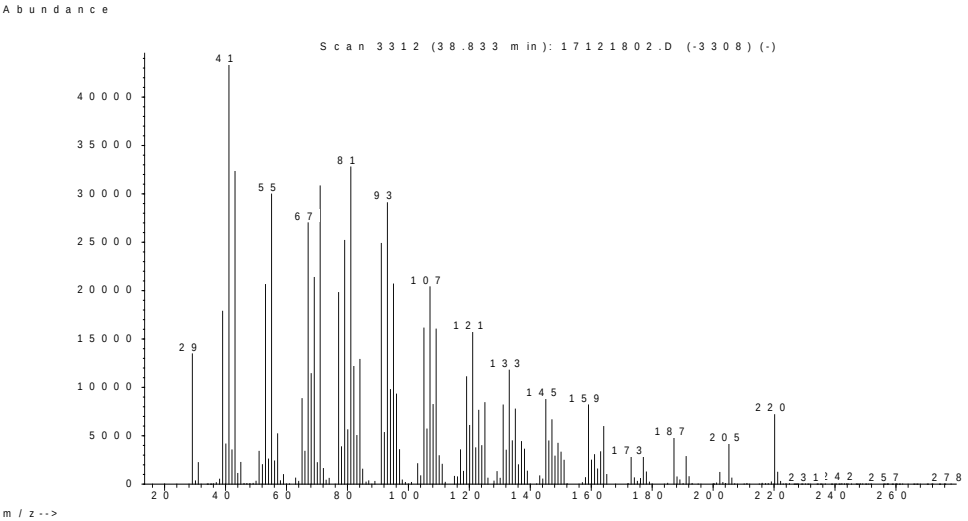

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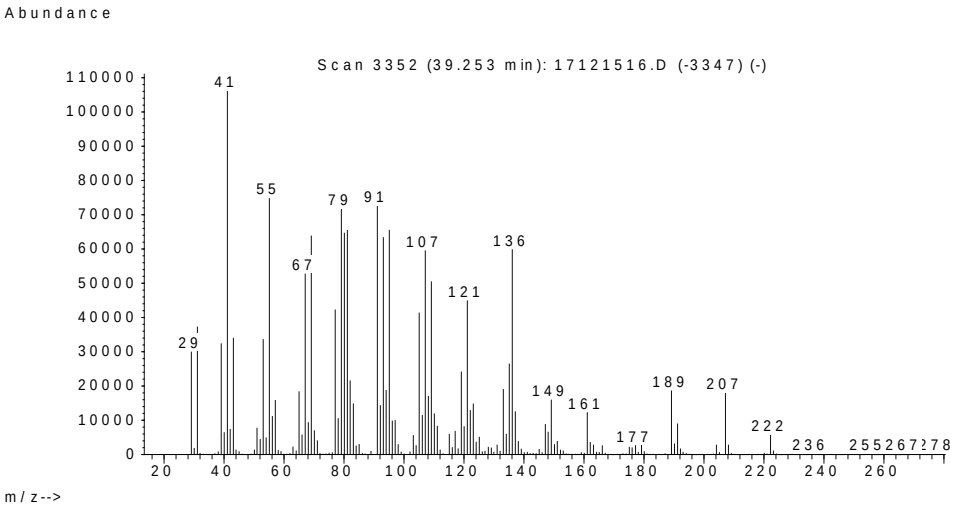

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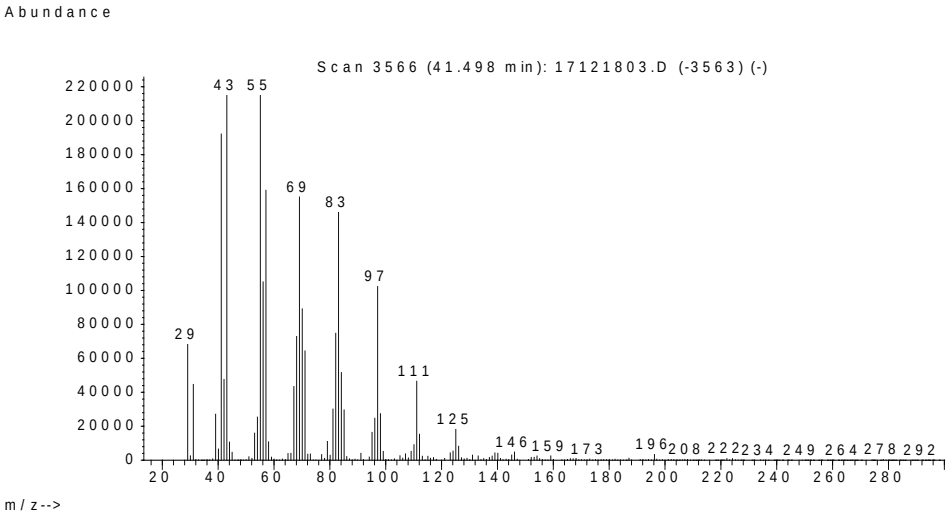

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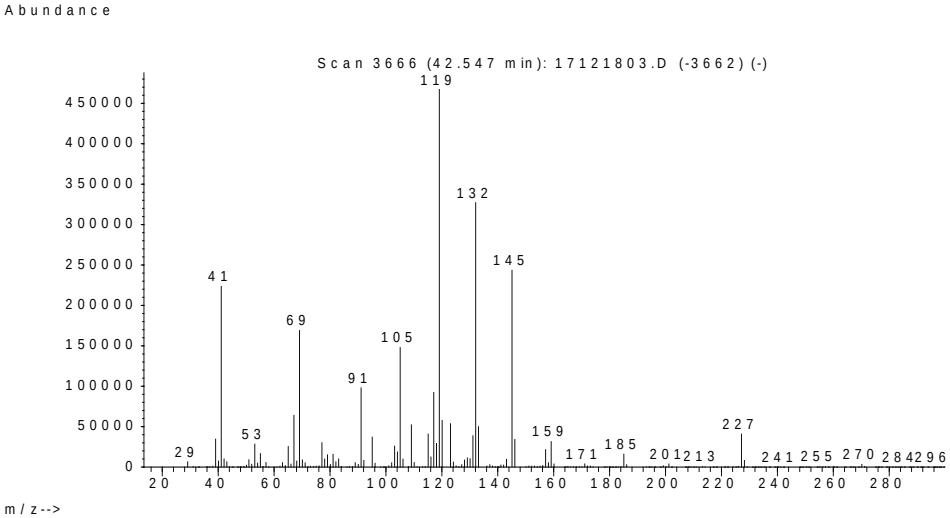

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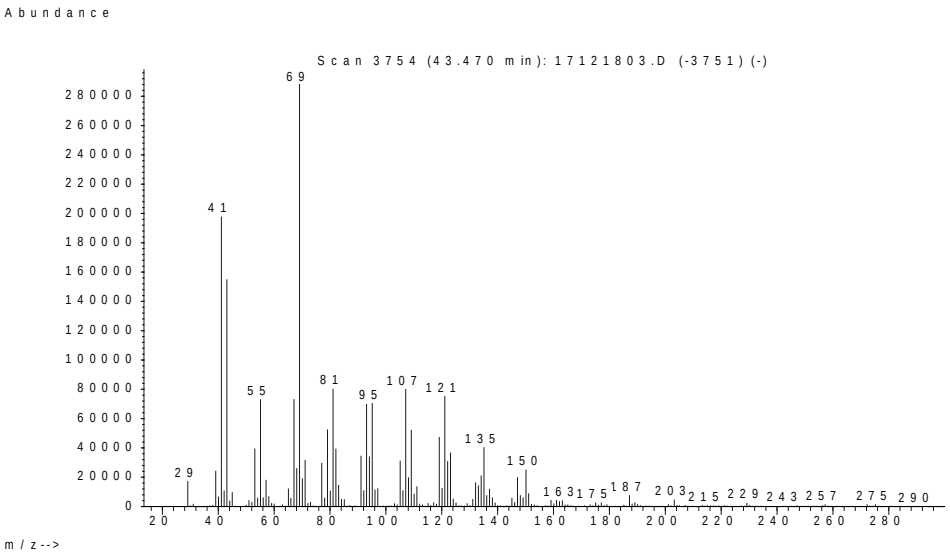

S2.12: Unknown compound 11