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IN VITRO CYTOTOXIC POTENTIAL ON HUMAN CANCER CELLS AND CHEMICAL CONSTITUENTS OF *Gryllotalpa orientalis*

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The mole cricket belongs to the *Insecta*, *Orthoptera*, *Gryllotalpide*, *Gryllotalpa* and is an agriculture pest. There are more than 30 species of mole crickets worldwide with six species in China, *G. orientalis*, *G. unispina*, *G. formosana*, *G. jinxiuensis*, *G. henana,* and *G. gryllotalpa*. The *G. orientalis* and the *G. unispina* have been used in medicine. The mole cricket, whose Chinese name is Lougu, is a traditional Chinese medicine with a long history, which was used to treat various hydroncus, to relieve the bowels, for urine movement, and to treat sores, and it had anti-inflammatory activities [1, 2]. New medicinal uses of *G. orientalis* to treat abdominal dropsy in the late stage of liver cancer [3], urinary retention [4, 5], calculus in the urinary system [6], and cancer in the digestive system [7] have been discovered recently. But the chemical constituents and pharmacological effects of active parts and compounds of Lougu have not been reported. Therefore, understanding the *in vitro* cytotoxic potential on human cancer cells and the chemical constituents of *Gryllotalpa orientalis* is important to provide a scientific basis for clinical application, quality evaluation, and further studies on *G. orientalis.*

Compound 1. White solid. EI-MS *m/z*: 167, 153, 140, 139, 125, 111, 98, 97, 84, 83, 70, 69, 57, 55, 54, 43. IR (KBr, cm^{-1}): 3452, 3005 (-C=C-), 2956, 2942, 2854, 1657, 1466, 1402, 1377, 1304, 1180, 1144, 1078, 1022, 972, 889, 721. Compared with the NIST chemical substance bank, it was identified as *trans*-9-tricosene.

Compound 4. Colorless oil. MW: 356. IR (KBr, cm^{-1}): 380.69, 2925.15, 2854.24, 1736.82, 1462.41, 1379.01, 1179.02, 1117.74, 1052.92, 723.11. EI-MS *m/z*: 265, 264, 221, 176, 151, 137, 112, 111, 98, 83, 69, 55, 41. Compared with the standard spectrum [8], the compound is 9-octadecenoic acid (*Z*)-, 2,3-dihydroxypropyl ester.

Compound 5. Colorless oil. MW: 228. EI-MS *m/z*: 229, 228, 199, 186, 185, 171, 166, 157, 143, 130, 129, 116, 115, 98, 97, 85, 83, 73, 71, 60, 57, 55, 45, 43, 41. Compared with the NIST chemical substance bank standard substance and TLC with benzene–chloroform 6:1, it was identified as tetradecanoic acid.

Compound 6. Colorless oil. MW: 254. EI-MS *m/z*: 254, 236, 194, 192, 165, 152, 138, 123, 111, 98, 97, 96, 83, 70, 69, 56, 55, 54, 43, 41. Compared with the NIST chemical substance bank standard substance and TLC with benzene–chloroform 6:1, it was identified as 11-hexadecenoic acid.

Compound 7. Colorless oil. MW: 256. EI-MS *m/z*: 257, 256, 227, 213, 199, 185, 172, 171, 158, 157, 143, 129, 115, 98, 97, 96, 87, 83, 74, 73, 71, 69, 60, 55, 45, 43. Compared with the NIST chemical substance bank standard substance and TLC with benzene–chloroform 6:1, it was identified as hexadecanoic acid.

Compound 8. Colorless oil. MW: 282. EI-MS *m/z*: 283, 282, 265, 264, 222, 220, 180, 151, 138, 127, 125, 111, 110, 98, 97, 95, 84, 83, 81, 71, 69, 57, 55, 54, 43, 41. Compared with the NIST chemical substance bank standard substance and TLC with benzene–chloroform 6:1, it was identified as octadec-9-enoic acid.

Compound 9. Colorless oil. MW: 284. EI-MS *m/z*: 285, 284, 255, 242, 241, 227, 213, 199, 185, 171, 157, 143, 129, 115, 98, 97, 96, 83, 74, 73, 71, 60, 57, 43. Compared with the NIST chemical substance bank standard substance and TLC with benzene–chloroform 6:1, it was identified as octadecanoic acid.

Compound 10. Colorless oil. MW: 312. EI-MS *m/z*: 283, 270, 269, 255, 241, 227, 213, 199, 185, 171, 157, 143, 129, 115, 98, 97, 85, 83, 73, 71, 57, 55, 43, 41. Compared with the NIST chemical substance bank standard substance and TLC with benzene–chloroform 6:1, it was identified as arachic acid.

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TABLE 1. Results of Cytotoxicity Experiment on QGY7703

Experimental drug	A_{595}	Cytotoxity, %	Experimental drug	A_{595}	Cytotoxity, %
Solvent 1	0.750±0.010		Solvent 3	0.767±0.042	
1	0.735±0.007	2.00	**7**	0.690±0.031*	10.04
2	0.743±0.027	0.90	**8**	0.751±0.087	2.09
3	0.750±0.013	0	**9**	0.733±0.032	4.43
Solvent 2	0.471±0.017		Solvent 4	1.187±0.047	
4	0.450±0.072	4.46	**10**	1.143±0.146	3.71
5	0.467±0.031	0.85	**11**	1.063±0.081*	10.47
6	0.480±0.035	0	**12**	1.167±0.057	1.68
			Colchicine	0.500±0.026**	57.88

Concentration: 30 μg/mL, colchicine: 6.4 μg/mL.
Compared with control: *P < 0.05, **P < 0.01.

TABLE 2. Results of Cytotoxicity Experiment on HL-60

Experimental drug	Concentration, μg/mL	A_{570}	Cytotoxity, %
Control		0.390±0.035	
	8.0	0.367±0.023	7.692
7	40.0	0.340±0.026	12.821
	200.0	0.210±0.017**	46.154
	1.6	0.373±0.015	4.359
11	40.0	0.310±0.010*	20.513
	1000.0	0.230±0.020**	41.026
Colchicine	1.60	0.220±0.017**	43.590

Compared with control note: *P < 0.05, **P < 0.01.

Compound 11. Colorless needle crystals. MW: 386. EI-MS *m/z*: 387, 386, 368, 353, 301, 275, 255, 247, 214, 213, 199, 173, 161, 159, 145, 119, 107, 93, 81, 69, 57, 55, 43, 41. Compared with the NIST chemical substance bank standard substance and TLC with petroleum–ethyl acetate 3:10, it was identified as holest-5-en-3-ol.

Compound 12. Colorless needle crystals. MW: 398. EI-MS *m/z*: 399, 398, 365, 355, 337, 301, 300, 273, 271, 255, 215, 213, 199, 161, 159, 133, 107, 95, 83, 81, 69, 55, 41. Compared with the NIST chemical substance bank standard substance and TLC with petroleum–ethyl acetate 3:10, it was identified as ergosta-5,22-dien-3-ol.

Compound 13. Colorless needle crystals. MW: 400. EI-MS *m/z*: 401, 400, 382, 367, 315, 289, 273, 255, 231, 213, 199, 163, 161, 145, 109, 107, 95, 81, 71, 57, 55, 43. Compared with the NIST chemical substance bank standard substance and TLC with petroleum–ethyl acetate 3:10, it was identified as campesterol.

Compound 14. Colorless needle crystals. MW: 412. EI-MS *m/z*: 414, 413, 412, 394, 351, 300, 271, 255, 253, 213, 173, 161, 159, 133, 121, 97, 95, 83, 81, 69, 55, 41. Compared with rhe NIST chemical substance bank standard substance and TLC with petroleum–ethyl acetate 3:10, it was identified as stigmasterol.

Compound 15. Colorless needle crystals. MW: 414. EI-MS *m/z*: 415, 396, 381, 329, 303, 273, 255, 231, 213, 178, 163, 161, 145, 119, 107, 95, 81, 69, 57, 55, 43, 41. Compared with the NIST chemical substance bank standard substance and TLC with petroleum–ethyl acetate 4:1, it was identified as sitosterol.

Compound 17. Colorless flat crystals, tested with TLC, colorless with 10% sulfuric acid. IR (KBr, cm^{-1}): 3063.59, 3032.46, 2924.16, 2734.82, 2655.58, 1699.12, 1498.26, 1454.25, 1407.98, 1340.02, 1242.47, 1197.07, 1074.49, 1029.84, 926.13, 839.18, 751.60, 700.16. EI-MS *m/z*: 93, 92, 91, 89, 77 (Ar), 73, 66, 65 (Ar), 63, 62, 51 (Ar), 46. Compared with the Sadtler Reference Spectra [8], the compound was identified as phenyl-acetic acid.

Cytotoxic Activity of the Extracts to Cancer Cells. The results are shown in Tables 1 and 2.

The results of cytology indicated that compounds **7** and **11** had significant cytotoxic effects on QGY7703 cells, while the other parts had no significant effects. The experiment with HL-60 cells using different concentrations of compounds further demonstrated that compounds **7** and **11** had a remarkable cytotoxic effect on QGY7703 cells, with compound **7** being more effective than compound **11**.

Materials. *G. orientalis* was obtained from the medicinal material market in Bozhou city, Anhui Province, China. The specimen were stored at the School of Life Sciences, Fujian Agriculture and Forestry University (No. 2004435).

Extraction and Isolation of Compounds. A 10.0 kg *G.orientalis* was extracted five times with 95% alcohol by heating and back-flowing for three times. A total of 2043.25 g dry powder was obtained from different extractions. The powder was dissolved in water and extracted with petroleum, ethyl acetate, and *n*-butanol, which gave 1819.25 g, 10.0 g, and 214.0 g, respectively. A 250 g portion of the petroleum extractants was analyzed in a silica gel column with petroleum–EtOAc in gradient elution and identified by TLC: compounds **1** (petroleum), **4** (petroleum–ethyl acetate 20:1), **5–10** (petroleum–ethyl acetate 10:1), and **11–15** (petroleum–ethyl acetate 5:1).

The ethyl acetate extractants (10.0 g) were isolated in a silica gel column (mobile phase petroleum–acetone 5:1 in gradient elution). Compound **17** was obtained using petroleum–acetone 4:1.

Experiments on Cytotoxic Activity. Drug and reagents. Each experimental compound was dissolved in 100% alcohol, which was diluted with RPMI1640 culture medium before further analysis. MTT (Serva products) was dissolved in PBS to 5 mg/mL and filtered to remove bacteria. Isopropyl alcohol was diluted with PBS to 10% (w/v) solution.

The crystal violet dye solution contained 0.5 g crystal violet, 8 mL of 40% methanal, 0.17 g NaCl, and 2.3 mL of alcohol. A final volume of 100 mL was obtained by adding double-distilled water.

The crystal violet extract solution contained 0.897 g trisodium citrate, 162.5 μL of 12 N HCl, and 47.5 mL of 100% alcohol. A final volume of 100 mL was obtained by adding double-distilled water.

Positive Control. A 7.9888 g portion of colchicine (Sigma) was dissolved in double-distilled water to 50 mL (0.4 mol/L) and then diluted to 400 mmol/L.

Experimental Cells. HL-60 and QGY7703 cells (provided by United Gene Company) were cultured in RPMI1640 (Sigma), which contained 10% NCS.

Experimental Materials. These included the 95% alcohol extracts, the petroleum extracts, the ethyl acetate extracts, the *n*-butanol extracts from *G. orientalis*, and other parts isolated from the petroleum extracts.

Cytotoxicity on QGY7703. Cell cytotoxicity was determined by the crystal violet dye method. 100 μL QGY7703 cells (1×10^4 cells/mL) were added in each well in a 96-well plate and incubated in a CO_2 incubation box at 37°C for 24 h. After incubation, the experimental compound or drug was added into each well for 48 h and dyed for 0.5 h with crystal violet. The plates were washed, 100 μL of crystal violet extract was added, and the absorbance was recorded at A_{595} in an enzyme-linked immunodetector.

Cytotoxicity on HL-60 Cells. 100 μL HL-60 cells (1×10^4 cells/mL) were added in each well in a 96-well plate. The diluted experimental compound or drug was then added to three plates as three replicates. The control was cell culture liquid that did not contain any drug. After incubation in a CO_2 incubation box at 37°C for 24 h, the number of cells was determined using the MTT method. After the cells were cultured for 4 h, 10 μL MTT was added, the supernatant was removed, 100 μL 10% isopropyl alcohol was added, the mixture was placed in the dark for 30 min, and the absorbance at A_{570} was recorded.

Calculations. The percent cytotoxicity was calculated using the formula Cytotoxicity % = $A_{solvent}–A_{drug}/A_{solvent} \times 100$, where A_{drug} and $A_{solvent}$ are absorbance values at A_{570} with or without cell culture liquid, respectively.

Statistical analysis of the data was performed using a two-tailed Student's t test. Results were considered significant if P values were <0.05 and ≤ 0.01.

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