Supporting Information

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Chemical Composition, Antibacterial, Synergistic Antibacterial and Cytotoxic Properties of the Essential Oil from *Gelsemium elegans* (Gardner & Champ.) Benth.

Youyi Tang[1], Xinyu Hu[1], Fan Xu[2] and Xiang Xing*[1]

[1] Marine College, Shandong University, Weihai, 264209, P. R. China
[2]Sdu-Anu Joint Science College, Shandong University, Weihai, 264209, P. R. China

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Experimental Section

S1: *Isolation and GC-FID and GC-MS Analysis of GE-EO*

The aerial parts of *G. elegans* (800 g) were subjected to hydrodistillation for 3.5 h using a Clevenger device. The distilled oil was then dried with anhydrous sodium sulfate and stored at a temperature of 4 °C until it was analyzed.

GC/FID analysis (Agilent 7890A gas chromatograph equipped with an HP-5MS silica column 30 m × 0.25 mm with 0.25 μm fixed phase) was used to quantify *G. elegans* essential oil components. GC/MS analysis was performed on an Agilent 7890A coupled to an Agilent 5975C mass detector. The temperature was set from 60 (held for 1 min) to 230 °C (held for 14 min) with an increase of 8 °C/min. Carrier gas (helium) was injected at 1.3 mL/min, with a split ratio of 50:1. The injector and detector temperatures were 250°C and 230°C, respectively. The injection volume was 0.2 μL. MS conditions: electron impact mode (EI, 70 eV); mass range: 50 to 550 m/z. The components of GE-EO were determined by comparing the mass spectra with the NIST library and the retention indices (RI) for n-alkanes (C_7-C_{30}) with those reported in the literature [1-3], under the same GC conditions.

S2: *Antibacterial Activity Assay*

The antibacterial activity of GE-EO was determined against four bacterial strains: *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), according to previously described methods [4]. For evaluation of the MIC (minimum inhibitory concentration) of GE-EO, the bacteria grown on MH agar at 37°C for 24 hours were diluted to 10^6 CFU/ml and mixed with a serial of two-fold dilutions of the EO (diluted in 5% dimethyl sulphoxide) in a 96-well plate. After incubation of the plate at 37 °C for 24 h, the MIC of GE-EO was measured for the minimum concentration of GE-EO that produced no visible bacterial growth. A solution (100 μL) from the MIC plates without color change was plated on MH agar and incubated at 37°C for 24 hours. MBC (minimum bactericidal concentration) was measured as the lowest concentration without bacterial growth.

S3: *Synergistic Effect of GE-EO with Conventional Antimicrobials*

The synergistic activity between GE-EO and antimicrobial agents (chloramphenicol and streptomycin) was performed by checkerboard assay in 96-well plates [5]. Briefly, 50 μL of two-fold serial dilutions of antibiotics and GE-EO, respectively, were added to each of a 96-well plate, with the final concentration ranging from 1/64 to 4 times the MIC. A final concentration of 1×10^6 CFU/mL of diluted bacterial suspension was added to each well. The plate was incubated at 37°C for 24 h. The combination effects of GE-EO and antimicrobials were expressed as the Fractional Inhibitory Concentration Index (FICI) using the following formula:

$$\text{FICI} = \frac{\text{combination MIC of GE-EO}}{\text{alone MIC of GE-EO}} + \frac{\text{combination MIC of antibiotic}}{\text{alone MIC of antibiotic}} \quad (1)$$

S4: *Cytotoxicity Assay*

The GE-EO was screened for *in vitro* cytotoxic activities against four human cancer cells (lung cancer cells A-549, colon cancer cells HCT-116, liver cancer cells HepG2, breast cancer cells MCF-7) and a non-cancerous cell HL-7702 using the MTT assay. Cytotoxicity is expressed as 50% growth inhibition percentage (IC_{50}). The cytotoxicity of GE-EO was tested as described in a previous report [5].

S5: *Statistical Evaluation*

Data were statistically analyzed using IBM SPSS software (version 27.0) (IBM Corp., Armonk, NY, USA) and GraphPad Prism 9.0. The results are considered statistically significant at $p < 0.05$ using Student's t-test. All the experiments were carried out in triplicate.

S6: References

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[5] R. L. Wang, Y. Gao and X. Xing (2020). Analysis of chemical composition and assessment of antioxidant, cytotoxic and synergistic antibacterial activities of essential oils from different plant parts of *Piper boehmeriifolium*, *Chem. Biodivers*. **17**, e2000245.