Supporting Information

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Chemical Composition, Antibacterial and Cytotoxic Activities of the Essential Oil from *Ficus tikoua* Bur.

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S1: Experimental Details

S1.1: Essential Oil Extraction

F. tikoua was collected from Guizhou Province of China in September 2018. Dry whole plant was obtained by air-dried in the dark at room temperature. The dry whole plant of *F. tikoua* was cut into pieces and placed in a Clevenger-type apparatus and submitted to hydrodistillation (4 h). Essential oil were dried over anhydrous Na_2SO_4, filtered and stored at 4°C.

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

Analysis of the essential oil was carried out by an Agilent 6890 gas chromatograph system coupled to a flame ionization detector (FID) and equipped with a HP-5MS capillary column (60 m × 0.25 mm × 0.25 μm film thickness). The injection volume was 2 μL (split ratio at 1:10) with helium as the carrier gas (flow rate: 1 mL/min). GC oven temperature was kept at 70°C for 2 min then increased to 180°C at 2°C per min, and programmed to 310°C at a rate of 10°C/min and then finally kept at 310°C for 14 min. The injector temperature was set at 250°C.

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

The GC-MS analysis was performed on Agilent 6890 GC system fitted with an Agilent 5975C MS and equipped with HP-5MS fused silica column. 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 amu.

S1.4: Identification of Chemical Constituents

The percentage of chemical component of the essential oil was calculated by the peak area normalization method. The constituents of the essential oil were identified by their retention time, retention indices relative to n-alkanes (C_8–C_{30}), and as well as by comparison of their mass spectra with those listed in literature, NIST 14 and Wiley 275 databases [1-6].

S1.5: Antibacterial Activity Test

The antibacterial test were performed with six strains of *Enterococcus faecalis* (ATCC 19433), *Staphylococcus aureus* (ATCC 6538P), *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (CICC 10389) and *Proteus vulgaris* (ACCC 11002).

Antibacterial activities of the essential oil and palmitic acid were assessed using the disc agar diffusion method with minor modification [7]. The essential oil and palmitic acid solutions were prepared by diluting with ethyl acetate (100 mg/mL). Filter paper discs (6 mm in diameter) were impregnated with 20 μL of the sample solution 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 result of antibacterial activity test is given in Table S1.

The MIC and MBC values of the essential oil and palmitic acid were determined by the microplate dilution method with slight modification [8]. The essential oil and palmitic acid were initially diluted in DMSO, and later in Mueller-Hinton Broth. The 100 µL of twofold dilution of tested sample 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 5×10^5 CFU/mL. The 96-well plates were incubated for 24 h at 37°C. 10 µL of resazurin aqueous solution (0.01%) was added to 96-well plates as an indicator of microbial growth by detecting the reduction of blue dye resazurin to pink resorufin. The 96-well plates were incubated at 37°C for 2 h in the dark. The MIC was defined as the lowest concentration of sample when the resazurin color changed. To obtain the MBC value, 10 µL samples were obtained from the wells (no color change) and subcultured in Mueller-Hinton agar. The MBC was determined as the lowest concentration without any microbial growth after 24 h at 37 °C [9]. Each test was repeated in triplicate. The result of antibacterial activity test is given in Table 2.

Table S1. Diameter of the inhibition zones of *F. tikoua* essential oil and palmitic acid using the disc agar diffusion method

Microorganisms	Diameter of the inhibition zones (mm)[a]		
	Essential oil	Palmitic acid	Streptomycin
Gram positive			
Enterococcus faecalis ATCC 19433	9.19 ± 1.03	8.24 ± 0.08	8.44 ± 0.37
Staphylococcus aureus ATCC 6538P	10.59 ± 0.36	7.12 ± 0.45	16.83 ± 0.78
Bacillus subtilis ATCC 6633	10.33 ± 1.81	6.75 ± 0.18	17.07 ± 2.08
Gram negative			
Pseudomonas aeruginosa ATCC 9027	9.17 ± 1.07	8.88 ± 0.34	11.52 ± 3.32
Escherichia coli CICC 10389	9.79 ± 1.52	8.31 ± 0.36	15.05 ± 0.71
Proteus vulgaris ACCC 11002	7.89 ± 0.49	8.06 ± 0.57	15.71 ± 0.77

[a]The diameter of the inhibition zones (mm) were measured including the diameter of the disk (6 mm). The sample solution: Essential oil and palmitic acid were diluted with ethyl acetate, at a concentration of 100 mg/mL, (tested volume: 20 µL); Positive control: Streptomycin (tested volume: 20 µL, 100 µg/mL).

S1.6: Cytotoxic Activity Test

Human lung adenocarcinoma (A549), non-small cell lung cancer (NCI-H1299), prostatic carcinoma (PC-3), leukemic (K562) cell lines, and normal human fetal lung fibroblasts cell line (MRC-5) were maintained in RPMI 1640 medium (2 mM glutamine, 10% fetal bovine serum, 100 U/mL penicillin and 100 U/mL streptomycin) and incubated in a humidified incubator at 37°C with 5% CO_2 atmosphere. The cytotoxic activity was evaluated by MTT assay with slight modification [10]. 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 oil and palmitic acid were dissolved in DMSO, and afterwards serially double diluted with culture medium. The diluted sample solution (20 µL) was added to each well and incubated for 24, 48

and 72 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 oil and palmitic acid inhibiting cell growth by 50% (IC_{50}). All experiments were performed in triplicate. The result of cytotoxic activity test is given in Table 3 in main text.

S1.7: 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.

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