

Supporting Information

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Chemical Composition, Antioxidant and Antimicrobial Activities of Essential Oil from the Leaves of *Lindera fragrans* Oliv.

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S1: Isolation and identification of the essential oil

Air-dried leaf of *L. fragrans* (500g) were subjected to hydrodistillation for 3 hours using a Clevenger-type apparatus according to the Chinese Pharmacopoeia [1] until the oil was no further increase in the volume. Hydrodistillation were repeated 3 times. The collected oil were dried over anhydrous sodium sulfate and stored in glass flasks at a temperature of -4°C until GC-MS analysis.

GC and GC/MS conditions

Gas Chromatographic analysis of *Lindera fragrans* Oliv. essential oil were carried out on Thermo Trace Gas Chromatograph (HP6890, USA) equipped with a DB-1 fused-silica capillary column (30 m × 0.25 mm, 0.25 µm film thickness) and fitted to FID detector. The oven temperature was programmed from 150°C to 280°C at 5°C/min. The temperature of injector and detector was kept at 280°C and 250°C, respectively. The injection volume was 0.5 µL of 1% solution prepared in n-hexane. Keep Helium flow rate 32 cm/s, split ratio 10:1.

Analyses of the essential oil were achieved on Thermo Trace Gas Chromatograph fitted with a DB-1 fused-silica capillary column (30 m × 0.25 mm, 0.25 µm film thickness), and equipped with a Polaris-Q External ion Trap Mass Spectrometer (HP6890-HP5973, USA). The oven temperature programme and injection volume were described in GC-FID. The GC-MS analysis parameters were the following: keep Helium flow rate 1.0 mL/minute, injection was performed at 250 °C in a split ratio 10:1; EI source 70eV/m/e, scan range 30-550AMU.

The constituents of the essential oil were identified based on their relative indices (RI) comparative to series of reference n-alkanes (C₈-C₃₀). Further identification was made by comparison of their mass spectra with those contained in the NIST2008 mass spectral library or with mass spectra from literature [2], and wherever applicable, by co-injecting pure standards. The relative concentrations of the chemical components of essential oil were obtained by normalization of the peak areas.

S2: Antimicrobial Activity

Four bacterial strains obtained from West China School of Pharmacy, Sichuan University, including *Escherichia coli* (ATCC, 25922), *Staphylococcus aureus* (ATCC, 25923), *Pseudomonas aeruginosa* (ATCC, 27853) and *Candida albicans* (ATCC, 10231) were used in the antimicrobial activity assay by the agar disc diffusion method. The solution of 0.1g/mL were prepared for the test sample and the reference substance (Amoxicillin-Kunming baker NORTON pharmaceutical co., LTD, China) by dissolving at PBS buffer. PBS buffer was used as negative control. Filter paper discs of 6.0 mm diameter were placed on the surface of culture dish, which were soaked with the essential oil. Each test was repeats three times. After 24h of incubation at 37°C (Bacterial) or 30°C (Fungi), the diameters of the inhibition zones were measured.

S3: Antioxidant Activity

DPPH assay

The efficacy of the essential oils to scavenge 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals was evaluated using a spectrophotometry method [3]. A 2 ml volume of different concentrations of tested samples was mixed with 1 mL of 0.15 mmol/L DPPH ethanol solutions. The mixture was vortexed and incubated at ambient temperature in the dark for 0.5h and the absorbance was recorded at 517 nm. Butylhydroxyanisole (BHA) and trolox were used as positive controls. All experiments were repeated three times. The inhibition percentages were measured using I% below.

$$I \% = (A_0 - A_S) / A_0 \times 100\%$$

Where, A_S is the absorbance of the tested sample, and A_0 is the ethanol solution of DPPH.

ABTS assay

The antioxidant ability of the oil was assessed by the ABTS (2, 20-azinobis-3-ethylbenzothiazoline-6-sulphonate) method [4]. The ABTS solution was made by mixing 7 mM ABTS solution and 2.45 mM ammonium persulfate then the mixture was incubated for 16 h in dark at 37 °C. BHT and trolox were used as positive control. The essential oil was diluted in DMSO properly and the ABTS solution was diluted with PBS before to obtain an initial absorbance of about 0.700 (± 0.02) at 734nm. Different concentrations of oils with 0.3 mL mixed with 3 mL of ABTS solution. The solution with methanol and ABTS without sample was used as a blank. All experiments were repeated three times. The inhibition percentages were measured using $I\%$. below.

$$I \% = (A_0 - A_S) / A_0 \times 100\%$$

Where, A_S is the absorbance of the tested sample, and A_0 is the PBS solution of ABTS.

β -Carotene bleaching assay

The antioxidant ability of the oil was also assessed by the β -Carotene bleaching assay [5]. The β -carotene was dissolved chloroform to obtain the concentration of 0.1mg/mL. And 1 ml of the solution was pipetted into a boiling flask together with Tween 40 (1 g) and linoleic acid (20 mg). The chloroform was removed by a rotary evaporator at 40°C and following add water (50 mL) immediately to obtain an emulsion (A). The emulsion was prepared in each experiment. A 0.2 ml volume of different concentrations of tested samples was mixed with 5 mL emulsion (A) in a test tube. The blank sample contained 0.2 ml of ethanol and 5 ml of the emulsion (A). Ethanol (200 μ l) was added to 5 ml of the emulsion (B) which contained Tween 40 (1 g), linoleic acid (20 mg) and 50 ml water. All the samples were placed in a water bath at 50°C for 2h. The absorbances were measured at 470 nm using spectrophotometry. The BHT and trolox were used as positive control. All experiments were repeated three times. The inhibition percentages were measured using $I\%$. below.

$$I \% = [(A_{S(2h)} - A_{0(2h)}) / (A_{0(0h)} - A_{0(2h)})] \times 100\%$$

Where, $A_{S(2h)}$ is the absorbance of the tested sample at moment of 2h, $A_{0(0h)}$ and $A_{0(2h)}$ is the absorbance blank sample at moment of 0h and 2h, respectively.

S4: References

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