SUPPLEMENTARY MATERIAL

Essential Oil Composition and Antibacterial Activity of *Lindera nacusua* (D. Don)

Merr.

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Abstract:

This study represents the first report on the chemical composition and biological activity of the essential oils from the leaves of *Lindera nacusua* (D. Don) Merr.. Twenty-two compounds were identified and quantified, and the major components of the oil were Caryophyllene oxide (8.79%), Hexahydrofarnesyl acetone (6.83%), β -Selinene (5.02%), Neophytadiene (4.53%), Palmitic acid (4.42%), Phytol (4.36%), α -Copaene (3.89%), 4a, 5, 8, 8a- β -Tetrahydro-2, 4, 5-trimethyl-1, 4-naphthalindione (3.83%), α -Cadinol (3.17%), Bulnesol (2.65%), and 1, 4-Dimethyl-7-(1-methylethyl) azulene (2.38%). The in vitro antibacterial activities of the essential oils were evaluated by the disc diffusion method. The inhibition zones against *Staphylococcus aureus* and *Candida albicans* were 23.7 and 23 mm. So the essential oil of *L. nacusua* showed potent antibacterial activity and potential high selectivity against *Staphylococcus aureus* and *Candida albicans*.

Keywords: *Lindera nacusua*; essential oil; GC/MS; antibacterial activity

Experimental

Materials and methods

Plant material: Leaves of *L. nacusua* were collected from Emeishan, Sichuan Province, China during May 2013, and were identified by Associate Professor Song LK. (School of Life Science and Engineering, Southwest Jiaotong University). Avoucher specimen (RM002) was deposited at the Natural Products Chemistry Laboratory, Southwest Jiaotong University.

Isolation of essential oil: The dried leaves of *L. nacusua* were submitted to hydrodistillation for 3 hours in a Clevenger-type apparatus according to the Chinese Pharmacopoeia. At the end of each distillation the oil were collected, dried with anhydrous Na₂SO₄, measured, and transferred to glass flasks that were filled to the top and kept at a temperature of -4°C for further analysis.

Gas Chromatographic (GC): Gas Chromatographic analysis was performed with a 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). The chromatographic conditions were as follows: The oven temperature increased from 50°C to 280°C at a rate of 5°C/min. The injector and detector temperatures were 280°C and 250°C, respectively. Helium used as the carrier gas was adjusted to a linear velocity of 32 cm/s. The samples were injected using split sampling technique by a ratio of 1:30. Quantitative data was obtained from electronic integration of peak areas without the use of correction factors.

Gas chromatography-Mass spectrometry (GC-MS): GC-MS analysis was performed with a Thermo Trace Gas Chromatograph equipped with a Polaris-Q External ion Trap Mass Spectrometer (HP6890-HP5973, USA). A DB-1 fused-silica capillary column (30 m × 0.25 mm, 0.25 µm film thickness) was used with a Helium carrier gas at 0.8 ml/minute; split mode (1:30); 0.1 µl as injection volume; 250°C as injection temperature. The oven temperature programme is described in section Gas

chromatography. An ionisation mode with electronic impact at 70 eV over a scan range of 20-450 atomic mass units was used.

Data is processed by HP MSD Chemistry Station, and then follows the peak area normalization method to calculate the percentage of part of the chemical composition of the volatile oil. The constituents of the essential oils were identified by comparing their retention indices relative to n-alkanes (C8–C20). Individual compounds were identified by comparing their recorded mass spectra with those stored in the NIST2008 mass spectral library of the GC-MS.

Screening of antibacterial activity: Four test organisms, one Gram-positive (*Staphylococcus aureus*, ATCC 25923), two Gram-negative (*Escherichia coli*, ATCC 25922; *Pseudomonas aeruginosa*, ATCC 27853) bacterial and one fungi (*Candida albicans*, ATCC 10231) strains, were used to assay the biological activity. These strains were purchased from West China School of Pharmacy, Sichuan University. Antibacterial activity of the essential oil was assayed using the agar disc diffusion method (Srivastava et al. 2001). A sample (0.1 ml) of a suspension of the tested micro-organism (10^8 cells/ml) was spread onto the surface of Mueller-Hinton agar plates. The concentration of the test sample and the reference substance all is 0.1g/ml. Filter paper discs (6.33mm in diameter, 20 µl in water absorption) were placed on the surface of inoculated plates, and then they were soaked with 0.2 mg of the essential oil. Amoxicillin (Shijiazhuang Pharmaceutical Group Co., Ltd., China) was used as positive controls. After incubation at 37°C (Bacterial) or 30°C (Fungi) for 24 hours, the diameters of the inhibition zones were measured in millimetres. Each test was carried out in triplicate.

Reference

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