Supporting Information

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Composition and Antimicrobial Activity of Essential Oils from Leaves and Twigs of *Magnolia hookeri* var. *longirostrata* D.X.Li & R. Z. Zhou and *Magnolia insignis* Wall. in Ha Giang Province of Vietnam

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S.1. Hydrodistillation of Essential Oils

In this process a total of 1 kg of each of the shredded leaf and twig samples of *M. hookeri* var. *longirostrata* and *M. insignis* were used for the hydrodistillation. A weighed sample was separately introduced into a 5 L flask and distilled water was added until it covers the sample completely. Hydrodistillation was carried out with a Clevenger-type distillation unit designed according to the specification as previously described [1] to obtain essentiel oils. The distillation time was 3.5 h and conducted at normal pressure. The volatile oils distilled over water and were collected separately into clean weighed sample bottles. The oils were kept under refrigeration (4^0C) until the they were tested and analysed. Each distillation was done in triplicate.

S.2. Analysis of Essential Oils

Analysis of the essential oils was performed by Gas chromatography/Mass spectrometry - Flame ionization detection (GC/MS-FID). GC/MS analysis was carried out using an Agilent GC7890A system with Mass Selective Detector (Agilent 5975C). A HP-5MS fused silica capillary column (60 m × 0.25 mm i.d. × 0.25 μm film thickness) was used. Helium was the carrier gas with a flow rate of 1.0 mL/min. The inlet temperature was 250 °C and the oven temperature program was as follows: 60 °C to 240 °C at 4 °C/min. The split ratio was 100:1 and the injection volume was 1 μL. The MS interface temperature was 270 °C, MS mode, E.I. detector voltage 1258V, and mass range 35–450 Da at 1.0 scan/s. FID analysis was carried out using the same condition with a HP-5MS fused silica capillary column (60 m × 0.25 mm i.d. × 0.25 μm film thickness). The FID temperature was 270 °C. Essential oil constituents were identified by their relative retention indices, determined by co-injection of a homologous series of n-alkanes (C5–C30), as well as by comparison of their mass spectral fragmentation patterns with those stored on the MS library NIST08, Wiley09, HPCH1607 [2-3]. Data processing software was MassFinder4.0 [4]. Each analysis was performed in triplicate. Component relative concentrations were calculated based on area peak of FID chromatography without standardization.

S.3. Microbial Strains

The antimicrobial activity of the essential oils was evaluated using 1 strain of Gram-positive test bacteria *Staphylococcus aureus* (ATCC 13709), 1 strain of Gram-negative test bacteria *Escherichia coli* (ATCC 25922), and 1 strain of yeast *Candida albicans* (ATCC 10231).

Minimum inhibitory concentration (MIC) and median inhibitory concentration (IC_{50}) values were determined using 3 strains of Gram-positive test bacteria including *Staphylococcus aureus* (ATCC 13709), *Bacillus subtilis* (ATCC 6633), and *Lactobacillus fermentum* (VTCC N4), 3 strains of Gram-negative test bacteria including *Salmonella enterica* (VTCC), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 15442), and 1 strain of yeast *Candida albicans* (ATCC 10231).The ATCC strains were obtained from American Type Culture Collection, The VTCC strains were obtained from Vietnam Type Culture Collection – Vietnam National University, Hanoi.

S.4. Screening of Antimicrobial Activity

The agar disk diffusion method was performed to test the antimicrobial activity of essential oil [5-6]. Testing media included Mueller-Hinton Agar used for bacteria and Sabouraud Agar used for fungi. Microorganisms were stored at −80°C and activated by culture medium prior to testing to reach concentration of 1.0 × 106 CFU/mL. A 100 μL inoculum solution was taken and spread evenly over the surface of the agar. Four holes were made on agar plates (about 6 mm in diameter each hole) using an aseptic technique. A total of 50 μL of each of original essential oil and two-fold diluted oil was put into each two holes using a pipette. The petri dishes were kept at room temperature for 2 to 4 hours and then incubated at 37°C for 18 to 24 hours. The presence or absence of growth around each antimicrobial disk on each plate culture was observed. The diameters of inhibition growth zones values were measured using a ruler with millimeter markings. The zone of inhibition is the point at

which no growth is visible to the unaided eye. An inhibition zone of 14 mm or greater (including diameter of the hole) was considered as high antibacterial activity [7-8].

Minimum inhibitory concentration (MIC) and median inhibitory concentration (IC_{50}) values were measured by the microdilution broth susceptibility assay [9,10]. Stock solutions of the oil were prepared in dimethylsulfoxide (DMSO). Dilution series were prepared from 8192 µg/mL to 2 µg/mL (2^{13}, 2^{12}, $2^{11,}$ 2^{10}, 2^{9}, 2^{7}, 2^{5}, 2^{3}, 2^{1} µg/mL) in sterile distilled water in micro-test tubes, from where they were transferred to 96-well microtiter plates. Bacteria grown in double-strength Mueller-Hinton broth or double-strength tryptic soy broth, and fungi grown in double-strength Sabouraud dextrose broth were standardized to 5×10^5 and 1×10^3 CFU/mL, respectively. The last row, containing only the serial dilutions of sample without microorganisms, was used as a negative control. Sterile distilled water and medium served as a positive control. After incubation at 37°C for 24 h, the MIC values were determined at well with the lowest concentration of agents completely inhibit the growth of microorganisms. The IC_{50} values were determined by the percentage of microorganisms inhibited growth based on the turbidity measurement data of EPOCH2C spectrophotometer (BioTeK Instruments, Inc Highland Park Winooski, USA) and Rawdata computer software (Belgium) according to the following equations:

$$\%\ \text{inhibition} = \frac{OD_{control(+)} - OD_{test\ agent}}{OD_{control(+)} - OD_{control(-)}} \times 100\%$$

$$IC_{50} = High_{Conc} - \frac{(High_{Inh\%} - 50\%) \times (High_{Conc} - Low_{Conc})}{(High_{Inh\%} - Low_{Inh\%})}$$

Where:
OD: optical density; control (+): only cells in medium without Antimicrobial agent; test agent: coresponds to a known concentration of Antimicrobial agent; control (-): culture medium without cells.

$High_{Conc}$/Low_{Conc}: Concentration of test agent at high concentration/low concentration; $High_{Inh\%}$/$Low_{Inh\%}$: % inhibition at high concentration/% inhibition at low concentration).

Reference materials: Ampicillin for Gram-positive bacterial strains with MIC values in the range of 0.004 to 1.2 µg/mL, Cefotaxime for Gram-negative bacterial strains with MIC values in the range of 0.07-19.23µg/mL, Nystatine for fungal strains with MIC value of 2.8 µg/mL.

S.5. Antibacterial and Anti-yeast Activity

Essential oil extracts of *M. hookeri* var. *longirostrata* and *M. insignis* at concentrations of 100% and diluted 50% were used to screen the antimicrobial activity. The standard agar disk diffusion method [5-6] was performed against three test microorganisms including *Staphylococcus aureus* (Gr(+) bacteria), *Escherichia coli* (Gr(-) bacteria), and *Candida albicans* (yeast). The test results obtained after 18-24 hours after incubation are presented in table S1.

Table S1. Antibacterial and anti-yeast activity of essential oils from leaves and twigs of *M. hookeri* var. *longirostrata* (HG1919) and *M. insignis* (HG1932)

Essential oil samples		Diameter of inhibition zones (mm)					
		S. aureus		***E. coli***		***C. albicans***	
		100% oil	Oil diluted 50%	100% oil	Oil diluted 50%	100% oil	Oil diluted 50%
M. hookeri var. *longirostrata*	Leaves	14.0±1.4	12.5±0.7	29.5±0.7	22.5±0.7	13.5±0.7	10.5±0.7
	Twigs	9.5±0.7	6	14.0±1.4	6	10.5±0.7	6
M. insignis	Leaves	24.5±0.7	16.5±0.7	39.5±0.7	15.5±0.7	>40	>40
	Twigs	21.0±1.4	15.5±0.7	26.5±2.1	19.5±0.7	>40	>40

Essential oils from leaves and twigs of *M. insignis* exhibited very strong inhibition [7-8] against all three microorganism strains tested in this study, while the oil from leaves of *M. hookeri* var. *longirostrata* showed strong activity against only *E. coli* with inhibition zones of more than 14.0 mm.

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