

## Supporting Information

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### Composition and Antimicrobial Activity of Essential Oils from Leaves, Twigs and Ripe Fruits of *Magnolia grandis*

(Hu & W.C.Cheng) V.S.Kumar in Ha Giang Province of Vietnam

Chu T. T. Ha<sup>1,2\*</sup>, Le N. Diep<sup>1</sup>, Dinh T. T. Thuy<sup>3</sup>, Trinh N. Bon<sup>4</sup>, Le T. T.  
Cham<sup>5</sup> and William N. Setzer<sup>6,7</sup>

<sup>1</sup>*Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (VAST),  
18 Hoang Quoc Viet, Cau Giay, Ha Noi 10072, Vietnam*

<sup>2</sup>*Graduate University of Science and Technology, VAST, 18 Hoang Quoc Viet, Ha Noi 10072, Vietnam*

<sup>3</sup>*Institute of Natural Product Chemistry, VAST, 18 Hoang Quoc Viet, Ha Noi 10072, Vietnam*

<sup>4</sup>*Silviculture Research Institute, 46 Duc Thang, Bac Tu Liem, Ha Noi 10056, Vietnam*

<sup>5</sup>*Vietnam National University of Agriculture, Trau Quy, Giam Lam, Ha Noi 10056, Vietnam*

<sup>6</sup>*Aromatic Plant Research Center, 230 N 1200 E, Suite 100, Lehi, UT 84043, USA*

<sup>7</sup>*Department of Chemistry, University of Alabama in Huntsville, Huntsville, AL 35899, USA*

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### **S.1. Hydrodistillation of Essential Oils**

In this process a total of 1.6-2.5 kg of each of the shredded leaf, twig and ripe fruit samples of *M. grandis* 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 essential 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°C) until they were tested and analyzed. Each distillation was done in triplicate.

### **S.2. Determination of Physical Properties of Essential Oils**

Three physical properties were determined for the essential oil: relative density, refractive index, and optical rotation. The standard ISO 279:1998 was used to determine the relative density, the standard ISO 280:1998 for the refractive index, and the standard method ISO 592:1998 for optical rotation.

### **S.3. 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.4. 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<sub>50</sub>) 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.5. 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 × 10<sup>6</sup> 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<sub>50</sub>) 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 16,384 µg/mL to 2 µg/mL (2<sup>14</sup>, 2<sup>13</sup>, 2<sup>12</sup>, 2<sup>11</sup>, 2<sup>10</sup>, 2<sup>9</sup>, 2<sup>7</sup>, 2<sup>5</sup>, 2<sup>3</sup>, 2<sup>1</sup> 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<sup>5</sup> and 1 × 10<sup>3</sup> 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 the well with the lowest concentration of agents that completely inhibited the growth of microorganisms. The IC<sub>50</sub> 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:

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

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

Where:

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

High<sub>Conc</sub>/Low<sub>Conc</sub>: Concentration of test agent at high concentration/low concentration; High<sub>Inh%</sub>/Low<sub>Inh%</sub>: % inhibition at high concentration/% inhibition at low concentration).

Reference materials: Ampicillin for Gram-positive bacterial strains with IC<sub>50</sub> and MIC values in the ranges of 0.02 - 3.62 µg/mL and of 0.125 - 32.0 µg/mL, Cefotaxime for Gram-negative bacterial strains with IC<sub>50</sub> and MIC values in the range of 0.07 - 4.34 µg/mL and 0.5 - 32.0 µg/mL, Nystatine for fungal strains with IC<sub>50</sub> and MIC values of 1.32 µg/mL and 8.0 µg/mL.

## S.6. Antibacterial and Anti-yeast Activity

Essential oils of *M. grandis* 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 of incubation are presented in table S1.

**Table S1 :** Antibacterial and anti-yeast activity of essential oils from leaves, twigs and ripe fruits of *Magnolia grandis*

| Essential oil samples | Diameter of inhibition zones (mm) |                 |                |                 |                    |                 |
|-----------------------|-----------------------------------|-----------------|----------------|-----------------|--------------------|-----------------|
|                       | <i>S. aureus</i>                  |                 | <i>E. coli</i> |                 | <i>C. albicans</i> |                 |
|                       | 100% oil                          | Oil diluted 50% | 100% oil       | Oil diluted 50% | 100% oil           | Oil diluted 50% |
| Leaves                | 18.5±0.70                         | 14.5±0.70       | 24.0±1.41      | 21.0±1.41       | >40                | >40             |
| Twigs                 | 12.5±0.70                         | 11.5±0.70       | 15.5±0.70      | 12.5±0.70       | 26.5±0.70          | 25.5±0.70       |
| Ripe fruits           | 14.0±1.41                         | 12.5±0.70       | 17.5±0.70      | 12.5±0.70       | 25.5±0.70          | 24.5±0.70       |

Essential oils from leaves and ripe fruits of *M. grandis* exhibited very strong inhibition [7-8] against all three microorganism strains tested in this study, while the oil from its twigs showed strong activity against *E. coli* and *C. albicans* with diameters of inhibition zones of more than 14.0 mm.

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