Supporting Information

***Rec. Nat. Prod*. 17:3 (2023) 571-576**

Essential Oil Compositions and Antimicrobial Activity of the Leaves of *Alphonsea monogyma* Merr. & Chun and *Goniothalamus banii* B. H. Quang, R. K. Choudhary & V.T. Chinh fromVietnam

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S1. Collection of the leaves of *A. monogyma* and *G. banii*

Large quantity of the leaves of *A. monogyma* were obtained from plants cultivated in Pù Mát National Park (GPS: 19º51′1′′N; 104º38′7′′E) at elevation of 210 m, on August 2020, while *G. banii* were harvested at an elevation of 762 m on September 2020 from Pù Hoat Nature Reserve (GPS: 19º42′43′′N; 104º49′29′′E). The plants were identified by Dr. Le Thi Huong. Voucher specimens HNU 820 and HNU, respectively, were deposited in the plant specimen room, Vinh University. The samples were subjected to cleaning by handpicking to remove unwanted materials to obtained 2 kg each.

S2 : Isolation of Essential Oils

Two kilogram each of the leaves of *A. monogyma* and *G. banii* was used for the hydrodistillation experiment. Each of the collected plant material was divided into three parts to ensure that each of the hydrodistillation was repeated three times. The sample was separately introduced into a 5 L flask after which distilled water was added until it covered the sample completely. Essential oil was obtained by hydrodistillation which was carried out in a Clevenger-type distillation unit designed according to an established procedure as described in previous studies [1,2]. The distillation time was 3 hr and conducted at normal pressure. The volatile oils which distilled over water were collected separately by running through the tap in the receiver arm of the apparatus into clean and previously weighed sample bottles. The oils were kept under refrigeration (4ºC) until the moment of analyses. The experiment was conducted in triplicate. The essential oil yield (%) was calculated by mass (g) of the essential oils divided by the mass (g) of the leaves of the plants.

S3 : Analysis of the Essential Oils of *A. monogyma* and *G. banii*

The analysis of the chemical constituents of the leaves essential oil of *A. monogyma* and *G. banii* was achieved using GC and GC/MS. Gas chromatographic (GC) analysis was performed on an Agilent Technologies HP 7890A Plus Gas chromatograph equipped with a FID and fitted with HP-5MS column (Agilent Technologies, Santa Clara, California, USA) of dimension 30 m × 0.25 mm with a film thickness of 0.25 μm. The analytical conditions employed in the GC analysis were: carrier gas H_2 with flow rate of 1 mL/min, while both the injector temperature (PTV: programmable temperature vaporization) and detector temperature were maintained at 250 ºC and 260 ºC, respectively. The column was temperature programmed from 60 ºC, with a 2 min hold, to 220 ºC (10 min hold) at a rate of 4 ºC/min. The essential oil (1.0 μL; 10% *n*-hexane solution) was injected using a split mode with a split ratio of 10:1, at inlet pressure was 6.1 kPa. Quantification was done using the calibration curves generated from the analyses of representative standard compounds from each class.

An Agilent Technologies (Santa Clara, California, USA) HP 7890A Plus Chromatograph fitted with a fused silica capillary HP-5MS column (dimension 30 m × 0.25 mm; film thickness 0.25 μm) and interfaced with a mass spectrometer HP 5973 MSD was used for the GC/MS analysis. The GC conditions were the same as those reported above for the GC analysis. However, He was used as the carrier gas. The MS was operated at ionization voltage of 70 eV with emission current of 40 mA, with the acquisitions scan mass range of 35-350 amu at a sampling rate of 1.0 scan/s as described previously [1-4].

S4 : Identification of the Constituents of the Essential Oils

The identification of constituents of essential oils from the GC/MS spectra of *A. monogyma* and *G. banii* was performed on the basis of comparison of retention indices (RI Exp.) with reference to a homologous series of *n*-alkanes (C_6-C_{40}), under identical experimental conditions. In some cases, co-

injection with known compounds under the same GC conditions was employed. The mass spectral (MS) fragmentation patterns were checked with those of other essential oils of known composition in literature [5-7] as described recently [1-4].

S5 : Microbes

Seven standardized ATCC strains from laboratory stock cultures were used in the teste for the antimicrobial activity of the essential oils. The Gram-negative strains were *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). The Gram-positive strains were *Bacillus cereus* (ATCC 14579), *Staphylococcus aureus* (ATCC 25923) and *Salmonella enterica* (ATCC 13076). A strain of yeast, *Candida albicans* (ATCC 10231) was also used for the experiment. Testing media included Mueller-Hinton Agar (MHA) used for bacteria and Sabouraud Agar (SA) used for fungi.

S6 : Antimicrobial Activity Assays

The minimum inhibitory concentration (MIC) values were measured by the microdilution broth susceptibility assay as described previously [1,2,6, 8]. Stock solutions of the oil were prepared in dimethylsulfoxide (DMSO). Dilution series 16384 to 2 µg/mL (2^{14}, 2^{13}, 2^{12}, 2^{11}, 2^{10}, 2^{9}, 2^{7}, 2^{5}, 2^{3} and 2^{1} µg/mL) were prepared in sterile distilled water inside the micro-test tubes from where they were transferred separately to 96-well microtiter plates with each of the microbial strains. The plate was then incubated overnight at 37°C. One hundred microlitre of the microbial culture of an approximate inoculums size of 1.0 x 10^6 CFU/mL was added to all well and incubated at 37°C for 24 h. The last row, containing only the serial dilutions of the sample without microorganisms, was used as a negative control, while Streptomycin and Chloramphenicol were used as positive control against bacteria and while Nystatinewas used as antifungal standard. The MIC values were determined as the lowest concentration of the oil that completely inhibits the growth of microorganisms.

All experiments were performed in triplicate. After incubation at 37°C for 24 h, the MIC values were determined at well with the lowest concentration of agents completely inhibiting 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, United States) and Rawdata computer software (Belgium) according to the following equations:

$$\%\ inhibition = \frac{\text{ODcontrol}(+) - \text{ODtest agent}}{\text{ODcontrol}(+) - \text{ODcontrol}(-)} \times 100$$

$$IC_{50} = High_{conc} - \frac{(\text{Highinh\%} - 50\%) \times (\text{HighConc} - \text{Low Conc})}{(\text{Highinh\%} - \text{Lowinh\%})}$$

where OD is the optical density, control (+) is the only cells in medium without antimicrobial agent, test agent corresponds to a known concentration of antimicrobial agent, control (-) is the culture medium without cells, High Conc/Low Conc is the concentration of test agent at high concentration/low concentration, and High Inh%/Low Inh% is the % inhibition at high concentration/% inhibition at low concentration.

S7: Statistical Analysis

All results of chemical composition and antimicrobial experiments were repeated three times and are expressed as mean ± standard deviation (SD).

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