

Supporting Information

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Mosquito Larvicidal Activity on *Aedes albopictus* and Constituents of Essential Oils from *Manglietia dandyi* (Gagnep.) Dandy

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S1. Plants Collection

The leaves and fruits of *M. Dandyi* were collected from Vũ Quang National Park, Hà Tĩnh (GPS: 18°20'N 105°54'E), Vietnam, in August 2018. Botanical identification was achieved at the Botany Museum, Nghệ An College of Economics, Vietnam, where a voucher specimen, LDL719, was deposited.

S2. Hydrodistillation of Essential Oils

A total of 1 kg of each of the pulverized leaves and fruits of *M. danydi* were used for the experiment at different hydrodistillation. A weighed sample was separately and carefully introduced into a 5 L flask where distilled water was added until it covered the sample completely. Hydrodistillation was carried out in an all glass Clevenger-type distillation unit designed according to Vietnamese Pharmacopoeia [1] to obtain essential oils. The distillation time for each experiment was 3 h. The volatile oils normally distilled over water were collected 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 (4°C) until the moment of analyses.

S3. Analysis of the Oils

Gas chromatography (GC) analysis was performed on an Agilent Technologies HP 7890A Plus Gas chromatograph equipped with a FID and fitted with HP-5MS column (30 m x 0.25 mm, film thickness 0.25 µm, Agilent Technology). The analytical conditions were: carrier gas H₂ (1 mL/min), injector temperature, 250°C; detector temperature 260°C; column temperature programmed from 40°C (held 2 min isothermally) and risen to 220°C (10 min hold) at 4°C/min. Samples were injected by splitting and the split ratio was 10:1. The volume of the oil injected was 1.0 µL. Inlet pressure was 6.1 kPa. Each analysis was performed in triplicate. The relative amounts of individual components were calculated based on the GC peak area (FID response).

An Agilent Technologies HP 7890A Plus Chromatograph fitted with a fused silica capillary HP-5 MS column (30 m x 0.25 mm, film thickness 0.25 µm) and interfaced with a mass spectrometer HP 5973 MSD was used for the GC/MS analysis, under the same conditions as those used for GC analysis. The conditions were the same as described above with He (1 mL/min) as carrier gas. The MS conditions were as follows: ionization voltage 70eV; emission current 40 mA; acquisitions scan mass range of 35-350 amu at a sampling rate of 1.0 scan/s.

S4. Identification of the Constituents

The identification of constituents from the GC/MS spectra of *M. dandyi* was performed on the basis of retention indices (RI) determined with reference to a homologous series of *n*-alkanes (C₆-C₄₀), under identical experimental conditions. The mass spectral (MS) fragmentation patterns were checked with those of other essential oils of known composition and with those in the literature [2].

S5. Mosquito larvae

Adults of *A. albopictus* were collected in Hoa Khanh Nam ward, Lien Chieu district, Da Nang city (16°03'14.9"N, 108°09'31.2"E). Adult mosquitoes were maintained in entomological cages (40 x 40 x 40 cm) and fed a 10% sucrose solution and were allowed to blood feed on mice. Eggs hatching were induced with tap water. Larvae were reared in plastic trays (24×35×5 cm). The larvae were fed on dog biscuits and yeast powder in the 3:1 ratio. All stages were held at 25 ± 2°C, 65–75% relative humidity, and a 12:12 h light: dark cycle at the Center for Entomology and Parasitology Research, Duy Tan University.

S6. Larvicidal Test

Larvicidal activity of the essential oils from *M. dandyi* was evaluated according to previous method with slight modifications [3]. For the assay, aliquots of the essential oils dissolved in EtOH (1% stock solution) was placed in a 200-mL beaker and added to water that contained 20 larvae (fourth instar). With each experiment, a set of controls using EtOH was also run for comparison. Mortality was recorded after 24 h and again after 48 h of exposure during which no nutritional supplement was added. The experiments were carried out $25 \pm 2^\circ\text{C}$. Each test was conducted with four concentrations of four replicates (100, 50, 25 and 12.5 $\mu\text{g/mL}$). Permethrin was used as a positive control.

The mortality rate was calculated according to the formula

$$Mc = (Mo - Mt) / (100 - Mt) \times 100$$

Mo = mortality in the treated groups, Mt = mortality in the control group and Mc = calculated mortality

S7. Statistical Analysis

The data obtained were subjected to log-probit analysis [4] to obtain LC_{50} values, LC_{90} values, 95% confidence limits, and chi square values using XLSTAT v. 2018.5 (Addinsoft, Paris, France). For comparison, LC_{50} values were also determined using the Reed-Muench method [5].

References

- [1] Vietnamese Pharmacopoeia (2009). Medical Publishing House, Hanoi, Vietnam, pp. 234-236.
- [2] National Institute of Science and Technology (2011). Chemistry Web Book. Data from NIST Standard Reference Database 69.
- [3] WHO (2005). Guidelines for Laboratory and Field Testing of Mosquito Larvicides. WHO/CDS/WHOPES/GCDPP, Geneva, Switzerland.
- [4] D. Finney (2009). Probit Analysis, Reissue, Ed. ed; Cambridge University Press: Cambridge, UK.
- [5] L.J. Reed and H. Muench (1938). A simple method of estimating fifty per cent endpoints, *Am. J. Hyg.* **27**, 493-497.