

SUPPLEMENTARY MATERIAL

Chemical composition and insecticidal activity of the essential oil from *Helichrysum faradifani* endemic to Madagascar

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Abstract

Helichrysum faradifani (Asteraceae) is a perennial shrub growing in rocky and sandy places of Madagascar. The plant is used in the Malagasy traditional medicine as wound healing and disinfectant and for the treatment of syphilis, diarrhoea, cough and headache. Searching for natural products effective against mosquito-borne diseases, we analyzed the chemical composition of the essential oil from the aerial parts of *H. faradifani* by GC-MS and evaluated its insecticidal activity against 2nd, 3rd and 4th instar larvae of the lymphatic filariasis vector *Culex quinquefasciatus* by acute toxicity assays. The most sensitive were 2nd instar (LC₅₀=85.7 µl.L⁻¹) larvae. For the 3rd and 4th instar larvae the estimated LC₅₀ were 156.8 and 134.1 µl.L⁻¹, respectively. Monoterpene hydrocarbons (51.6%) were the prevalent fraction of the oil, with the bicyclic α-fenchene (35.6%) as the major component. Sesquiterpene hydrocarbons (34.0%) were the second group in the oil, with γ-curcumene (17.7%) as the most abundant component.

Keywords: *Helichrysum faradifani*, essential oil, GC-MS, insecticidal activity, *Culex quinquefasciatus*

3. Experimental

3.1. Plant material

Aerial parts of *H. faradifani* were collected in January 2015 at Vatomandry at the east coast of Madagascar (GPS: S/19°20'27''- E/48°56'33'' altitude 46 m a.s.l.). Botanical identification was made at the Departement of Botany of the Parc Botanique et Zoologique de Tsimbazaza Antananarivo, by comparison with an authentic specimen. A voucher specimen has been kept at the herbarium of the Institut Malgache de Recherches Appliquées under the accession code MAD-1070-b.

3.2. Hydrodistillation

The essential oil was obtained by using a portable alembic. Freshly collected aerial parts of *H. faradifani* (4 kg) were extracted by steam distillation for 3 h. The oil yield was calculated as 3.12 ml/kg.

3.3. GC/MS Analysis

GC-MS Analysis was performed on an Agilent 6890N gas chromatograph coupled to a 5973N mass spectrometer using a HP-5 MS (5% phenylmethylpolysiloxane, 30 m, 0.25 mm i.d., 0.1 µm film thickness; J &W Scientific, Folsom) capillary column. The temperature program was as follows: 5 min at 60 °C, subsequently 4 °C/min up to 220°C, then 11 °C/min up to 280 °C, held for 15 min, for a total run of 65 min. Injector and transfer line temperatures were 280 °C; He was used as the carrier gas, at a flow rate of 1 mL/min; split ratio, 1 :50; acquisition mass range, 29–400 *m/z*. Mass spectra were acquired in electron-impact (EI) mode with an ionization voltage of 70 eV. A mixture of aliphatic hydrocarbons (C8–C30; Sigma, IT-Milan) in *n*-hexane was directly injected into the GC under the above temperature program, in order to calculate the temperature-programmed RIs of peaks in the chromatogram. Oil sample was diluted to 1 : 100 in *n*-hexane, and the volume injected was 2 µL. Data were analyzed by using MSD ChemStation software (Agilent, Version G1701DA D.01.00). Major oil constituents were identified by co-injection with authentic standards purchased from Sigma-Aldrich (Milan, Italy). Otherwise, the peak assignment was carried out according to the recommendations of the International Organization of the Flavor Industry (IOFI, <http://www.iofi.org/>), i.e., by the interactive combination of chromatographic linear retention indices that were consistent with those reported in literature (Adams 2007; NIST 08 2008; FFNSC2 2011) for apolar stationary phases, and MS data consisting in the computer matching with the WILEY275, NIST 08 ADAMS, FFNSC 2 and a home-

made (based on the analyses of reference oils and commercially available standards) libraries.

3.4. Insecticidal activity

Early 2nd, 3rd and 4th instar mosquito larvae (*Culex quinquefasciatus* Say) were used in the study. The larvae were reared in the laboratory colony (Crop Research Institute, Prague, Czech Republic). The mosquito adults were provided with a 10% sucrose solution (w v⁻¹) and a 1-week-old chick for blood feeding. The larvae were fed on dog biscuits and yeast powder in the ratio 3:1.

Mosquito larvicidal trials were carried out according to WHO (1996) standard procedures, with slight modifications (Pavela 2015). The essential oil was diluted in dimethyl sulfoxide (DMSO) to prepare a serial dilution of the test concentrations. For experimental treatment, 1 mL of serial dilution was added to 224 mL of distilled water in a 500 mL-glass bowl and shaken gently to produce a homogeneous test solution. The *Cx. quinquefasciatus* larvae were transferred in water into a bowl of the prepared test solution, with final concentration 300.0; 200.0; 150.0; 100.0; 70.0 and 50.0 $\mu\text{L.L}^{-1}$ (25 larvae beaker⁻¹). Four duplicate trials (100 larvae per single replication) were carried out for every sample concentration, and for each trial, a negative control was included using distilled water containing the same amount of DMSO as the test sample. Mortality was determined after 24 h of exposure, during which no food was offered to the larvae.

3.5. Statistical analysis

Experimental tests demonstrated that more than 20% of the controlled mortality was discharged and repeated. When the controlled mortality reached 1–20%, the observed mortality was corrected by the Abbott's formula (Abbott, 1925) and an LC₅₀, LC₉₀ regression equation, and a 95% confidence limit was calculated using probit analysis (Finney, 1971).

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