SUPPLEMENTARY MATERIAL

Chemical composition of *Melicope belahe* (Baill.) T. G. Hartley (Rutaceae) leaf essential oil from Madagascar

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Melicope belahe (Baill.) T.G. Hartley (Family Rutaceae) is an endemic species to Madagascar. The chemical composition of leaf essential oil is reported for the first time. A sample was extracted by hydrodistillation and analysis was carried out by combination of chromatographic (GC), spectroscopic and spectrometric (MS, ^{13}C NMR) techniques. In total, 56 compounds have been identified. The chemical composition was dominated by α-pinene (42.6%) followed by linalool (6.2%) and (E)-β-caryophyllene (5.2%).

Keywords: *Melicope belahe*; leaf essential oil; Madagascar

Experimental

Plant collection

Leaves of *M. belahe* were collected in Joffre-Ville, Northern Madagascar, in November 2014. The plant was identified by Rakotonandrasana Stéphan Richard. A voucher specimen was deposited in the Centre National de Recherche Pharmaceutique (CNARP, Antananarivo, Madagascar) under the accession ST1497.

Botanical description

M. belahe is a tree, 12 to 15m high; thick boughs, heavily streaked-costulae (5-10mm diameter at the ends) young stems, petioles, petiolules and ribs of the underside of the leaflets, warty-puberulous. Leaves opposite, trifoliate, somewhat leathery, green and almost homochromes on dry; petiole 3 to 10cm; long, narrowly channeled above; petiolules 1 to 10 mm long , the longest median that side; somewhat uneven leaflets obovate side (7.5-12 x 4-5,3cm) non-equilateral corners at the base, obovate-cuneate also the median, but larger (12-16 x 6-7cm) and equilateral all rounded at the top, with no trace of cusp or acumen; secondary ribs (nervure or veins) more visible below than above, few (7-9 pairs); direct, visible dark dots on the underside of the leaf blade; by transparency , citrus-like dots very fine and very dense. Axillary or terminal panicles, in very shaded parts of rain forest, around 900 to 1000m; flowers with 4 stamens (Baillon 1886).

Extraction and fractionation of essential oil

The oil sample was isolated by hydrodistillation for 3h using a Clevenger-type apparatus from leaves (910 g). Yield, calculated on fresh mass basis (w/w), was 0.07%. *M. belahe* leaf oil (500 mg) was subjected to column chromatography (Silica gel 15 g, 63-200 µm) leading to a hydrocarbon fraction HF (387 mg) and an oxygenated fraction OF (95 mg).

Gas chromatography (GC)

Essential oil sample analysis was performed on Perkin-Elmer Clarus 500 gas chromatograph (FID) equipped two fused silica capillary columns (50 m x 0.22 mm, 0.25 µm film thickness) BP-1 (polydimethylsiloxane) and BP-20 (polyethylene glycol). The oven temperature was programmed as follow: 60-220°C at 2°C/min and then held isothermal at 220°C for 20 min. The carrier gas was hydrogen, at a flow of 1.0 mL/min; injector port and detector temperature were 250°C. Sample was injected by splitting and the split ratio was 1:60. The relative proportions of the oil constituents were expressed as percentages obtained by peak area

normalisation, Retention indices (RI) were determined relative to the retention times of a series of n-alkanes (C7-C28) with linear interpolation ("Target Compounds" software from Perkin-Elmer). The relative proportions of the oil constituents were expressed as percentages obtained by peak area normalisation without using correcting factors.

Mass spectroscopy (GC-MS)

GC-MS analyses were carried out using a Agilent Technologie 7890A detector (quadrupole), directly coupled to a Agilent Technologie 5975C, equipped with a fused-silica capillary column (30 m x 0.25 mm i.d., film thickness 0.25 µm), HP-MS 5% phenylmethylsiloxane. Carrier gas, helium at 1.0 mL/min; split, 1:80; injection volume, 0.2 µL. The injection port was set at 250°C; the oven temperature was programmed from 60°C to 250°C at 4°C/min. Significant quadrupole MS operating parameters: Ion source temperature, 150°C; electron impact ionisation at 70 eV with scan mass range of 33-350 *m/z*.

^{13}C Nuclear magnetic resonance (NMR)

^{13}C NMR analyses (essential oil and CC fractions) were performed on a Bruker AVANCE 400 Fourier Transform spectrometer operating at 100.623 MHz for ^{13}C, equipped with a 5 mm probe, in deuterated chloroform ($CDCl_3$), with all shifts referred to internal tetramethylsilane (TMS). ^{13}C NMR spectra were recorded with the following parameters: pulse width (PW), 4 µs (flip angle 45°); acquisition time, 2.73 s for 128 K data table with a spectral width (SW) of 22 000 Hz (220 ppm); CPD mode decoupling; digital resolution 0.183 Hz/pt. The number of accumulated scans was 3000 (50 mg of the mixture in 0.5 mL of $CDCl_3$). Exponential line broadening multiplication (1.0 Hz) of the free induction decay was applied before Fourier transformation.

Identification of components

Identification of individual components was based: (a) on comparison of their GC retention indices (RI) on polar and apolar columns, determined relative to the retention times of a series of *n*-alkanes with linear interpolation (Target Compounds software of Perkin-Elmer), with those of authentic compounds (b) on computer search using digital libraries of mass spectral data and comparison with published data (National Institute of Standards and Technology 1999, König et al. 2001, Adams 2007) (c) on comparison of the signals in the ^{13}C NMR spectra of essential oils and the two fractions of chromatography with those of

reference spectra compiled in the laboratory spectral library, with the help of a laboratory-made software (Tomi and Casanova 2006, Bighelli and Casanova 2009).

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