

SUPPLEMENTARY MATERIAL

Reinvestigation of carvotanacetone after 100 years along with minor terpenoid constituents of *Blumea malcolmii* Hook. F. essential oil

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Abstract

Reinvestigation of essential oil constituents of *Blumea malcolmii* Hook. F. (Asteraceae) was carried out after 100 years by using gas chromatography equipped with flame ionization detector (GC-FID) and gas chromatography coupled with mass spectrometry (GC/MS). Eighteen constituents, representing 99.2% of the total oil, were identified. The major compound was identified by GC/MS and NMR (^1H - and ^{13}C -) as carvotanacetone (92.1%). The minor compounds of essential oil have also been identified for the first time in *B. malcolmii*. The oil was found to be rich in oxygenated monoterpene type constituents (95.0%).

Keywords: *Blumea malcolmii* Hook. F.; Asteraceae; carvotanacetone; GC/MS; NMR (^1H - and ^{13}C -)

Experimental

Plant material

The aerial parts of *B. malcolmii* were collected in the month of February, 2014 from Tilari of district Kholapur (N 15.79952; E 74.17441), Maharashtra, India, at a height of ~760 m. The plant was identified by a Taxonomist, Dr. Harsha Hegde, Scientist, Regional Medical Research Centre (RMRC), Belgaum where a voucher specimen (RMRC-1304) has been deposited.

Isolation of essential oil

The fresh plant material (500 g) was hydro-distilled for 3 h using a Clevenger type apparatus. The oil was dried over anhydrous sodium sulfate and stored at $-4\text{ }^\circ\text{C}$ until further analysis. The yield of the oil was 0.27% v/w.

Isolation of compound

The essential oil of *B. malcolmii* (4.0 g) was chromatographed on a silica gel (230-400 mesh; Qualigens Fine Chemicals, Mumbai, India) column ($3.5 \times 100\text{ cm}^2$) eluted with increasing polarity mixtures of *n*-hexane/diethyl ether to give carvotanacetone (purity (99.71% determine by GC-Varian 450 gas chromatograph using the similar analytical conditions as reported in experimental section).

Analysis of the essential oil

The chemical composition of the oil was analysed by using a gas chromatograph (GC) (Varian 450 fitted with a fused silica capillary column TG-5, $30\text{ m} \times 0.25\text{ mm i.d.}$, $0.25\text{ }\mu\text{m}$ film thickness) under the experimental conditions reported earlier (Joshi, 2013b,c; 2014b). The oven temperature was programmed from 60 to $220\text{ }^\circ\text{C}$ at $3\text{ }^\circ\text{C}/\text{min}$, using nitrogen as

carrier gas. The injector and the detector (FID) temperature were set at 230 and 240°C, respectively. Gas chromatography–mass spectrometer (GC–MS) analysis was employed a Thermo Scientific Trace Ultra GC interfaced with a Thermo Scientific ITQ 1100 mass spectrometer fitted with a TG-5 fused silica capillary column (30 m × 0.25 mm; 0.25 μm film thickness). The oven temperature range was programmed from 60 to 220°C at 3°C/min and helium was used as carrier gas at 1.0 mL/min flow rate for analysis. The injector temperature was set at 230°C, and the injection volume was 0.1 μL in *n*-hexane, with a split ratio of 1:50. MS were taken at 70 eV with a mass range of *m/z* 40-450 and other parameters used were those reported earlier (Joshi, 2012, 2013d; 2014c).

Identification of the components

Identification of constituents were done on the basis of retention index (RI, determined with reference to homologous series of *n*-alkanes C₈-C₂₅, under identical experimental conditions), MS library search was done in NIST 08MS Library (Version 2.0 f. Thermo Fisher Scientific Austria), WILEY MS 9th Edition (Thermo Fisher Scientific Austria), and by comparing with the MS literature data (Adams, 2007) and NMR (¹H- and ¹³C-) of the major compound. The NMR spectral data was compared with the reported literature (Ross et al. 1997). The relative amounts of individual components were calculated based on the GC peak area (FID response) without using a correction factor.

¹H- & ¹³C-NMR data of the compound carvotanacetone

¹H- NMR (CDCl₃, 300MHz) □ ppm

□ 6.73 (1H, *d*, J=6.0 Hz, H-3), 2.05 (1H, *m*, H-4a), 2.15 (1H, *m*, H-4b), 1.82 (1H, *m*, H-5), 2.32 (1H, *m*, H-6a), 2.49 (1H, *dd*, J₁=15.9 Hz, J₂=3.6 Hz, H-6b), 1.77 (*s*, 3H, H-7), 1.54 (1H, *m*, H-8), 0.89 (6H, *d*, J=6.9 Hz, H-9, H-10).

¹³C- NMR (CDCl₃, 75 MHz) □ ppm

□ 200.8 (*s*, C-1), 135.2 (*s*, C-2), 145.4 (*d*, C-3), 29.8 (*t*, C-4), 42.0 (*d*, C-5), 41.9 (*t*, C-6), 15.6 (*q*, C-7), 32.0 (*d*, C-8), 19.5 (*q*, C-9), 19.4 (*q*, C-10).

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