# Characterization of volatile compounds in *Anemia tomentosa* var. *anthriscifolia* (Schrad.) Mickel *ex-vitro*

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*Anemia tomentosa* var. *anthriscifolia* (Schrad.) Mickel is an aromatic fern growing on islands of vegetation associated with rocky substrates. Its woody aroma is derived from a triquinane sesquiterpene-rich essential oil (1), which is also endowed with antimycobacterial activity against *Mycobacterium tuberculosis* (MIC 100µg $ml^{-1}$) (2). The major constituents of this essential oil were: silphiperfol-6-ene (14.7 %), (-)-epi-presilphiperfolan-1-ol (30.6 %), presilphiperfol-7-ene (3.9 %), cameroonan-7α-ol (4.4 %), prenopsan-8-ol (1.9 %) and presilphiperfolan-8-ol (8.3 %). Considering the ornamental, medicinal and fragrant potential of the species, our group indulged into the investigation of the chemical composition of this essential oil in relation to their triquinane sesquiterpenes (responsible for biological activity/aroma) upon *in vitro* multiplication of the plant. However, preliminary *in vitro* conditions imposed on the plant favored the production of monoterpenes instead of triquinane sesquiterpenes (1). In this study, a different protocol was used and we evaluated the contents of the major mono and sesquiterpenes present in the volatile fraction of germinated *in vitro* individuals in culture medium MS plus kinetin (0.5 µmol, 5 µmol) which were later acclimatized to the external environment (*ex vitro*). Plant's sporophytes were subjected to SDE extraction method (Simultaneous Distillation and Extraction), where small plant weights (± 5 g) are extracted in solvent dichloromethane. The samples were analyzed by GC/FID and GC/MS in Shimadzu GC-2010 systems, both with DB-5MS fused silica capillary columns (30 m X 0.25 mm X 0.25 µm). Hydrogen was used as carrier gas for GC/FID and helium for GC/MS, both with a flow rate of 1.0 mL $min^{-1}$. Oven temperature was raised from 60 to 290 °C at 3°C $min^{-1}$. Mass detector was operated in electronic ionization mode at 70 eV. The percentage composition was obtained by normalization from FID. Volatile components were identified by comparison of both mass spectra and linear retention indices with spectral library and literature. Each sample was injected 3 times in GC/FID. Monitored monoterpenes were *trans*-pinocarveol, *trans*-verbenol and pinocarvone. Their relative percentage levels in plants grown on MS medium without accretion of kinetin were respectively 1.4479 ± SD; 0.3029 ± SD and 1.2346 ± SD. In plants grown in MS + 0.5 µmol KIN, the levels were respectively 1.5226 ± SD; 0.3012 ± SD; and 1.1352 ± SD. In plants grown in MS + 5 µmol KIN, their levels were respectively 1.4110 ± SD; 0.2779 ± SD and 0.9916 ± SD. The sesquiterpenes monitored were the presilphiperfol-7-ene, silphiperfol-6-ene, α-guaiene and (-)-*epi*-presilphiperfolan-1-ol. Their respective levels in MS0 plants were 4.8473 ± SD; 19.8043 ± SD; 6.4682 ± SD; 27.7704 ± SD. In plants grown in MS + 0.5 µmol KIN levels were respectively 5.1114 ± SD; 21.1305 ± SD; 10.2357 ± SD; 25.8907 ± SD. For plants grown in MS + 5 µmol KIN, their levels were 4.6180 ± SD; 19.6240 ± SD; 9.2128 ± SD; 25.2812 ± SD. These results show that the in vitro protocol led to *ex-vitro* plants where the sesquiterpenes are the major volatiles.

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2. Pinto, S.C. et al. Nat. Prod. Commun., 2009, **4**, 1733-1736.

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