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# Establishment and volatiles composition of hairy root cultures of *Digitalis mariana* subsp. *heywoodii*

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Palavras-chave: Plantaginaceae, *Digitalis mariana* subsp. *heywoodii*, foxglove, hairy roots.

**Introdução.** Foxglove is the common name for the *Digitalis* genus members of the Plantaginaceae. Mostly known for being the source of therapeutically important cardiac glycosides, several species are also of ornamental value. *Digitalis mariana* subsp. *heywoodii* (P.Silva & M.Silva) Hinz (= *Digitalis purpurea* subsp. *heywoodii* P.Silva & M.Silva) is endemic of the Iberian Peninsula and has a restricted habitat distribution [1]. Viewing to study cardenolide as well as non-cardenolide compounds to access their bioactive properties, hairy root cultures from this rare species were obtained and their volatile composition was evaluated.

**Material e Métodos.** *D. mariana* subsp. *heywoodii* seedlings were grown aseptically from seeds, on solid Schulz medium [2]. Aseptic, 20-day-old seedlings were used for the establishment of hairy roots, by inoculation with *Rhizobium rhizogenes* strain A4 pRiA4::70GUS as detailed in [3]. Hairy roots emerging from the hypocotyl and epicotyls were excised and transferred to liquid, antibiotic- and growth regulator-free SH medium [in 3] and maintained in darkness at 24ºC on orbital shakers (80r.p.m.). Following establishment of the hairy root cultures in liquid SH medium a regular subculturing routine was used to maintain the culture. After every 3 weeks, a portion of the root clump was aseptically removed and transferred to fresh culture medium. At least one-year-old cultures maintained under regular routine subculture were accessed for volatiles composition. Volatiles were isolated by hydrodistillation and analysed by GC and GC-MS as in [3].

**Resultados e Discussão.** Hairy roots of *D. mariana* subsp. *heywoodii* were established successfully in the dark in liquid SH medium, showing high branching ability, fast growth and the typical "rooty" phenotype. Hexadecanoic acid (= palmitic acid, 54%), *cis*,*cis*-9,12-octadecadienoic acid (= linoleic acid, 16%) and linoleic acid ethyl ester (10%) were the main volatiles components. These *in vitro* cultures will be used for the propagation of this species as well as for fundamental studies on cardenolides and other phytochemicals formation and for bioactivity assays.

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