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Validated HPTLC method for determination of ledol and alloaromadendrene in the essential oil fractions of *Rhododendron tomentosum* plants and *in vitro* cultures and bioautography for their activity screening

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Abstract

Rhododendron tomentosum (*Ledum palustre*) is a bog shrub used in traditional medicine for treatment of respiratory and rheumatic diseases. Due to the large variability of the chemical composition of its essential oil, depending on the habitat, the *in vitro* cultures were established as the alternative source of the volatile fraction. There is a need to monitor a quality of the field grown as well as *in vitro* plant material, especially if potentially toxic aromadendrane derivatives are concerned.

In this study the HPTLC method was developed and validated for quantification of ledol and alloaromadendrene in *R. tomentosum* essential oils obtained from the plants collected in various locations as well as *in vitro* cultures.

For qualitative analysis, chromatograms were developed on HPTLC Si 60 plates at distance of 50 mm without preconditioning using hexane: ethyl acetate (9:1) and visualized with *p*-anisaldehyde reagent. For quantitative analysis, chromatograms were developed on HPTLC Si 60 plates at distance of 40 mm with 20 min preconditioning using hexane: ethyl acetate (9:1), visualized with vanillin / phosphoric acid reagent and subjected to densitometric detection (560 nm). The content of ledol and alloaromadendrene in different samples was determined using the validated HPTLC method (12 – 280 mg and 2 – 60 mg 100 g^{-1} dried plant material, respectively) and was compared with GC/MS results.

The bioautographic antioxidant HPTLC assays in DPPH and in riboflavin-light-NBT systems as