

**Seasonal variation in the volatile chemical profile of *Lithraea molleoides* (Vell.)
Engl. analyzed by SPME, with a comparative study of its essential oil
composition**

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Lithraea molleoides (Vell.) Eng. (Anacardiaceae), popularly known in Uruguay as “aruera”, is a 12-meter-high tree responsible for allergic contact dermatitis (1), being associated to non-volatile C₁₅/C₁₇ alkenyl-catechols (“urushiols”) (2). However, airborne allergy cases are also reported and, despite the cause behind has not been elucidated yet, it is likely the mediation of volatile compounds. As a continuation of our previous work on the volatile chemistry of *L. molleoides* (3,4), in this work we described the seasonal variation on the chemical profile assessed by SPME in georeferenced individuals of a defined tree population. This study was conducted sampling in Iporá locality, Tacuarembó (Northern Uruguay) from Spring 2022 to Autumn 2024. Simple and composed leaves from the lower part of three individuals were collected and transported immediately to the laboratory where classification and visual separation by colors (“new”: clear-green; “old”: dark-green) were performed. Healthy simple leaves or leaflets (0.5g) were extracted by SPME using a DVB/CAR/PDMS fiber at 50°C for 30 minutes. After extraction, the volatiles were directly desorbed (5 min) at the injection port of a GC/MS system Shimadzu GC-2010/QP2020. Two different stationary phases were employed: Rx1-MS (60 m × 0.25 mm × 0.25 µm) and Stabilwax-MS (30 m × 0.25 mm × 0.25 µm), with Helium (1.0 mL·min⁻¹) as carrier and a Split ratio of 50:1. For the two columns the oven program was 40°C (5 min)-5°C·min⁻¹-235°C (2 min). MS was programmed in electron ionization (70eV) scanning m/z from 50 to 350 uma. For comparison purposes (specifically to discard the presence of artifacts in the oil), the essential oil was extracted by steam-distillation (only in the case of the first collection) from 200g of leaves by a laboratory equipment (Figmay, Argentina). The oil was diluted 1:100 in *n*-hexane and injected in the same analytical conditions. The volatiles were identified by comparison with commercial mass spectral libraries (Wiley, FFNSC, NIST) and by calculation of LRIs, injecting in the same conditions a *n*-alkane solution (C₈-C₂₀). Volatile abundances were obtained directly by area normalization, without the use of correction factors. Some of the most abundant identified components were α - and β -pinene, myrcene, limonene, linalool, *trans*- β -caryophyllene, δ -cadinene, γ -murolene, β -selinene, α -guaiene, and *trans*-nerolidol. Significative quantitative differences were evidenced in the volatile profiles because of (1) extraction method (SPME or steam distillation), (2) seasonality, and (3) the age of the leaves (“new” or “old”); no influence of leaves’ morphology was observed. Some volatile compounds were found to be more abundant in ‘new’ leaves compared to ‘old’ ones, with γ -murolene standing out as the most prominent difference, consistently observed across all seasons. Many of the detected components have been reported as elicitors of allergy (5). Thus, the findings of this work suggest the key role of *L. molleoides* volatiles in airborne allergy.

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