

## CHEMICAL COMPOSITION OF CUTICULAR WAXES AND CUTIN IN EUCALYPTUS GLOBULUS LEAVES

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### ABSTRACT

Tree leaves have important physiological roles among which the dynamics of gas and water exchange with the environment. The external surfaces of leaves are specific frontiers that control these exchanges in which the epidermal cells and the cuticle play an important role. The cuticle includes epicuticular and intracuticular waxes composed by terpenes, sterols, fatty acids and alcohols, and a cutin matrix which is a glyceridic polymer of fatty acids and alcohols. The amount and chemical composition of cuticular waxes and cutin have been studied in leaves of different species, mostly using isolated cuticles with only a few cases with determinations made on the surface of whole leaves. The cuticle of *Eucalyptus globulus* has been studied in detail at anatomical and chemical levels in isolated cuticles. The waxes represented 37.3% of the cuticle and were composed by terpenes and terpenoids (45%), followed by phenolics (21%). Cutin represented 53.6 % of cuticle and was chiefly composed of aliphatic  $\omega$ -hydroxyacids (69%) and  $\alpha,\omega$ -diacids (8%) with a C18/C16 ratios of 3.1.

The objective of this work is to study the amount and composition of cuticular waxes and cutin of *E. globulus* using a whole leaf approach, with a critical analysis of the methodology taking into account its potential use as unit process to be integrated in a *E. globulus* based biorefinery. The biometric characteristics of the leaves will be determined in order to have results on surface area, leaf mass and leaf area index that will allow to express the results on a dry leaf mass basis ( $\mu\text{g}$  per gram) or as a leaf coverage ( $\mu\text{g}$  per  $\text{cm}^2$  of leaf area). The abaxial and adaxial surfaces of the leaves were observed by scanning electron microscopy.

The extraction of the cuticular waxes will be made using dichloromethane with a short contact time at ambient temperature to extract mostly the epicuticular waxes and with a 3 h extraction at near boiling point for extraction also of the intracuticular waxes. In the pre-extracted leaves, cutin will be depolymerized using a methanolysis procedure. The solubilized compounds of the cuticular waxes and the monomers obtained from the cutin depolymerization will be analysed by GC-MS after derivatization. The identified compounds are reported and grouped in the main chemical families present.

After cuticle chemical removal, the leaves are kept for further chemical analysis, namely including determination of extractives and cell wall structural components (lignin, cellulose and hemicelluloses). A potential integration of eucalypt leaves in a biorefinery-based path is proposed.

**Keywords:** cuticle, cutin, waxes, epiderm, leaves