## Supercritical fluid extraction of triterpenoids from Acacia dealbata biomass

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Acacia dealbata was introduced in the Portuguese coastal region for ornamental purposes, dunes erosion protection and wood supply [1]. Due to its fast growth, it is spread throughout Portugal mainland occupying 0.25 % of the total forest area [2]. It harms the natural flora by forming dense stands that hinder other species development. For the forest-depending sectors, this species control generates significant amounts of biomass (such as wood, bark, and leaves) which are usually burned for energy production. *A. dealbata* has been associated with traditional medicinal practises and is known to contain valuable triterpenoids (TTs), such as, lupenone, lupenyl acetate, lupeol,  $\alpha$ -amyrin and  $\beta$ -amyrin [3,4], to which bioactive properties (e.g., anti-inflammatory, antiviral, antidiabetic and anticancer [5,6]) have been attributed.

In this work, the bark and leaves of A. dealbata were extracted with supercritical fluid extraction (SFE) using carbon dioxide (SC-CO<sub>2</sub>, flow rate 12 g min<sup>-1</sup>) under different conditions of pressure (200-300 bar) and temperature (40-80 °C), with and without the addition of modifiers (ethanol and ethyl acetate), during 6 h. For comparison, conventional Soxhlet extractions using *n*-hexane, dichloromethane, ethyl acetate and ethanol were also performed. In both cases, the extracts were analyzed by gas chromatography coupled with mass spectrometry (GC-MS) to identify and quantify the main TTs individual yields and concentrations. The total extraction yields (n<sub>Total</sub>) ranged from 1.1 to 7.7 wt.% (bark) and 1.8 to 11.6 wt.% (leaves), for SFE (SC-CO<sub>2</sub>, 200 bar, 40 °C) and ethanol Soxhlet extraction, respectively. The addition of cosolvent and increase of pressure and temperature favoured  $\eta_{Total}$  while Soxhlet extraction achieved its best results with higher polarity solvents. The major TTs identified in bark extracts were lupenyl acetate and lupenone, with higher yields and concentrations being obtained by SFE. Harsher conditions (SC-CO<sub>2</sub> modified, higher pressure and temperature) enhance the TTs yield but are not the most suitable for the extraction of those two compounds. The main TTs identified in the leaves were lupenone,  $\alpha$ -amyrin,  $\beta$ -amyrin and  $\beta$ -amyrone. SFE selectively extracted only lupenone, achieving considerably higher individual yields and concentrations in comparison with Soxhlet although the remaining triterpenoids were extracted to a lower extent. As observed for bark, SFE harsher conditions increase triterpenoids extraction but selectivity becomes lower.

This work contributes to the valorization of *A. dealbata* biomass residues and provides insights on the potential of SFE for a future forestry-based biorefinery process.

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## References

- C.A. Kull, C.M. Shackleton, P.J. Cunningham, C. Ducatillon, J.-M. Dufour-Dror, K.J. Esler, J.B. Friday, A.C. Gouveia, A.R. Griffin, E. Marchante, S.J. Midgley, A. Pauchard, H. Rangan, D.M. Richardson, T. Rinaudo, J. Tassin, L.S. Urgenson, G.P. von Maltitz, R.D. Zenni, M.J. Zylstra, Divers. Distrib. 17 (2011) 822–836.
- [2] I. da C. da N. e das Florestas, Inventário Florestal Nacional (IFN6) Principais resultados, 2019.
- [3] C.S.D.D. Oliveira, P. Moreira, J. Resende, M.T. Cruz, C.M.F.F. Pereira, A.M.S.S. Silva, S.A.O.O. Santos, A.J.D.D. Silvestre, Int. J. Mol. Sci. 21 (2020) 1814.
- [4] F.B.M. Pereira, F.M.J. Domingues, A.M.S. Silva, Nat. Prod. Lett. 8 (1996) 97–103.
- [5] F. Xu, X. Huang, H. Wu, X. Wang, Biomed. Pharmacother. 103 (2018) 198–203.
- [6] M. Saleem, Cancer Lett. 285 (2009) 109–115.