

***Acacia dealbata* Link. biomass – a prospective source of valuable lupane triterpenoids using supercritical fluid extraction**

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1. Introduction

The genus *Acacia* comprises several species which are considered aggressive invaders in the Iberian Peninsula. Such is the case of *Acacia dealbata* Link., which is native to Australia and was introduced in Portugal for dunes erosion protection and ornamental purposes. This species is now widespread throughout the Portuguese forest landscape, occupying *ca.* 8000 hectares of total area¹. Its rapid growth after forest fires can prevent the local flora from developing and lead to productivity decline of forest-based industries. These issues have motivated periodical removals of this tree generating significant amounts of biomass². While the wood obtained can have multiple applications the remaining biomass, such as leaves and bark, is typically burned for energy production. Recently, several lupane-type triterpenoids were identified in these biomasses, namely lupenone and lupenyl acetate^{3,4}, which are associated with important bioactive properties, such as anti-inflammatory, anti-diabetes, anti-virus, among others⁵. This work aims the valorization of *A. dealbata* Link. residues (leaves and bark) using supercritical fluid extraction (SFE) for the production of lupane-type triterpenoids-rich extracts with potential pharmaceutical or nutraceutical applications, under the scope of biorefineries and the circular economy principles. Furthermore, the use of a green and innocuous extraction method, such as SFE with carbon dioxide as solvent, follows the green chemistry principles of preventing waste and use of benign solvents and auxiliaries^{6,7}.

2. Materials and Methods

The bark and leaves of *Acacia dealbata* Link. were extracted with supercritical CO₂ and the effects of pressure, temperature and cosolvent such as ethanol and ethyl acetate were tested. For every SFE, the CO₂ flow rate, time of extraction, and the particle size and amount of biomass were kept constant (see Table 1). Conventional Soxhlet extractions using dichloromethane were also performed.

Total extraction yields were determined by weighing. The extracts were analyzed by gas chromatography coupled to mass spectrometry (GC-MS) to identify the triterpenoids and to determine their individual yields and concentrations in the extracts.

Table 1. SFE experimental conditions tested.

Condition	Range
Pressure (bar)	100 – 300
Temperature (°C)	40 – 80
Cosolvent content (wt.%)	0 – 10

3. Results and discussion

The Soxhlet total extraction yields were 1.4 wt.% and 3.8 wt.% for bark and leaves, respectively, which demonstrate a higher amount of extractives present in the leaves. The SFE total extraction yields for leaves and bark ranged from about 50 to 90 % and from 5 to 100 % of the Soxhlet extraction yields, respectively. The main triterpenoids identified in the bark extracts were lupenyl acetate and lupenone, in this order. For the leaves, apart from the higher content of extractives, a wider diversity of compounds was obtained, namely, lupenone, β -amyrone, α -amyrone and β -amyrin.

The total triterpenoids yield for the bark reached a maximum of 1500 mg kg_{bark}⁻¹ while for the leaves it reached *ca.* 8000 mg kg_{bark}⁻¹. Despite the differences in total and triterpenoids extraction yields between the bark and leaves of *A. dealbata* Link., the content of the targeted triterpenoids in the extracts reached

an identical maximum of *ca.* 30 wt.%. The increase of pressure, temperature, and cosolvent content favored the total extraction yields of both biomasses. However, the optimum conditions for triterpenoids total yield and selectivity were found at a balance between pressure and temperature so that the solutes solubility could be increased without compromising the solvent power of the supercritical fluid. The addition of ethyl acetate and ethanol as cosolvent increases the total extraction yields, whereas the triterpenoids yield and content in the extracts were not improved in comparison with pure CO₂ SFE. Ethanol addition provided poorer results than ethyl acetate, which was able to match the triterpenoids yield and concentrations obtained with pure CO₂.

4. Conclusions

SFE was able to match the productivity of the conventional Soxhlet extraction method, reaching 90 % and 100 % of the reference yield for the leaves and bark, respectively. Moreover, it achieved better yields and selectivities for lupane triterpenoids. The effect of pressure and temperature were of utmost importance to tune the process towards the desired response whereas the addition of cosolvent (ethanol or ethyl acetate) only favored productivity at the cost of selectivity for triterpenoids. This work reveals the potential of supercritical fluid extraction for the valorization of *Acacia dealbata* Link. residues as a prospective source of added-value triterpenoids.

References

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