

Characterisation of bioactive protein-bound polysaccharides from *Amanita ponderosa* cultures

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Different bioactive compounds of edible mushrooms are responsible for their antioxidant, antitumor, antimicrobial, immunomodulatory, antiatherogenic and hypoglycemic reported properties [1, 2]. These properties are mostly due to the ability to synthesize different polysaccharides, namely protein-polysaccharide (PPS) complexes. The antioxidant capacity of these compounds present great interest in preventing innumerable diseases, including cancer, cardiovascular, auto-immunes diseases and accelerated aging. *Amanita ponderosa* are wild edible mushroom (Fig 1a₁, a₂, b), growing in some Mediterranean microclimates, namely in Alentejo region (Southern Portugal) and Andalusia (Southern Spain) [3], and establishes a mycorrhizal symbiosis with holm oaks and cork trees like *Quercus ilex* and *Q. suber*. There are few studies with respect to this species, however in this work was possible to obtain *A. ponderosa* pure cultures (Fig 1c, d₁, d₂) from strains collected in different areas of Alentejo.

This study focused on the characterisation of the PPS complexes produced in liquid cultures of *A. ponderosa*. Batch cultures (Fig 1e₁, e₂) were performed during 15 days, and polysaccharides concentrations were determined by the phenol-sulphuric method. A combined FTIR-ATR (Fourier-transform infrared using the attenuated total reflection) and Raman spectroscopy was used for the screening of bioactive PPS compounds present in the culture extracts. After identification of these bioactive compounds, PPS extracts were fractionated by size exclusion chromatography (SEC) using Sephacryl S-300 as stationary phase. Then, the chromatographic fractions and extracts were analysed by SEC, using an HPLC system coupled to UV (280 nm) and RI detectors in order to determine polysaccharide average molecular weights (Mw). The toxicity of